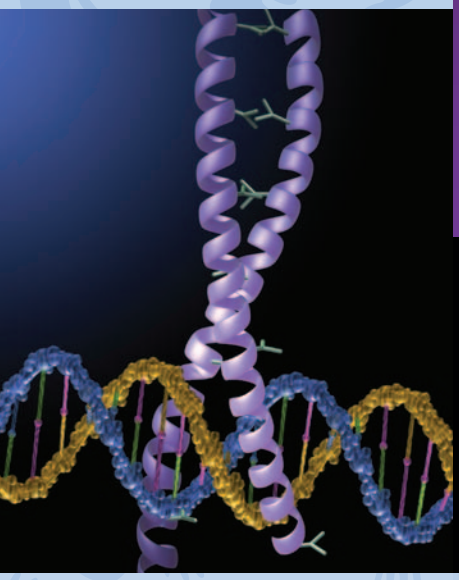

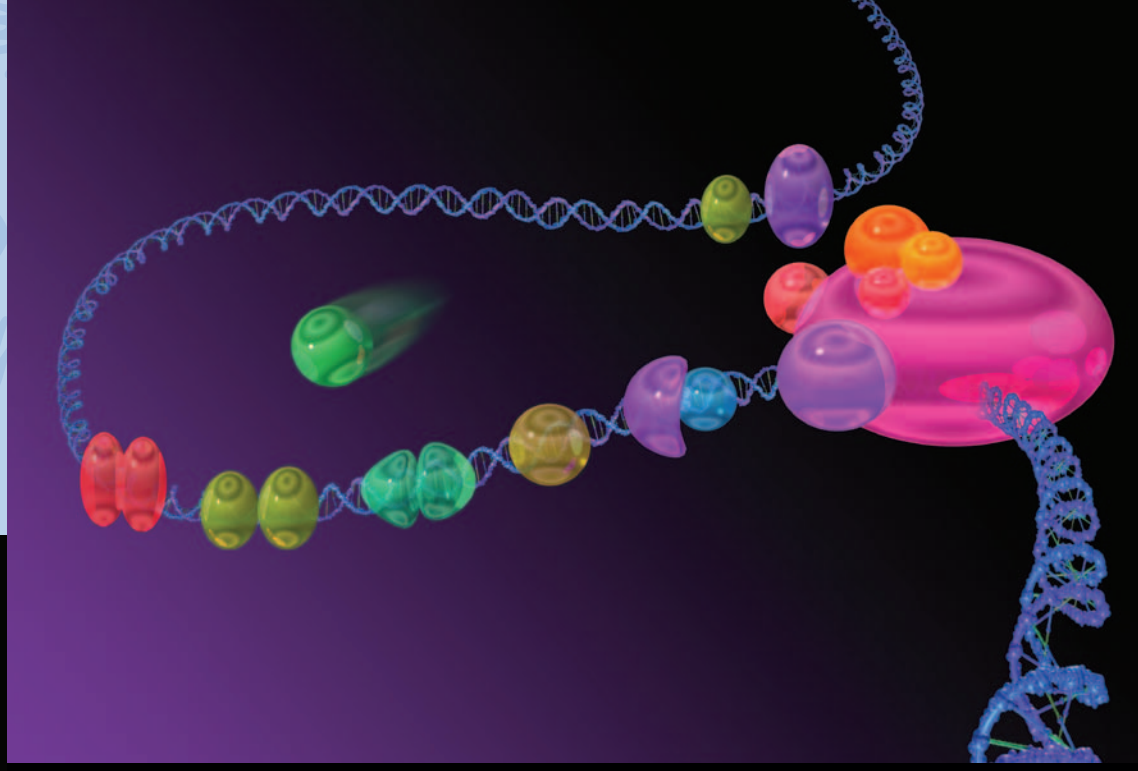

Thermo Scientific Pierce Protein:Nucleic Acid Interaction Technical Handbook

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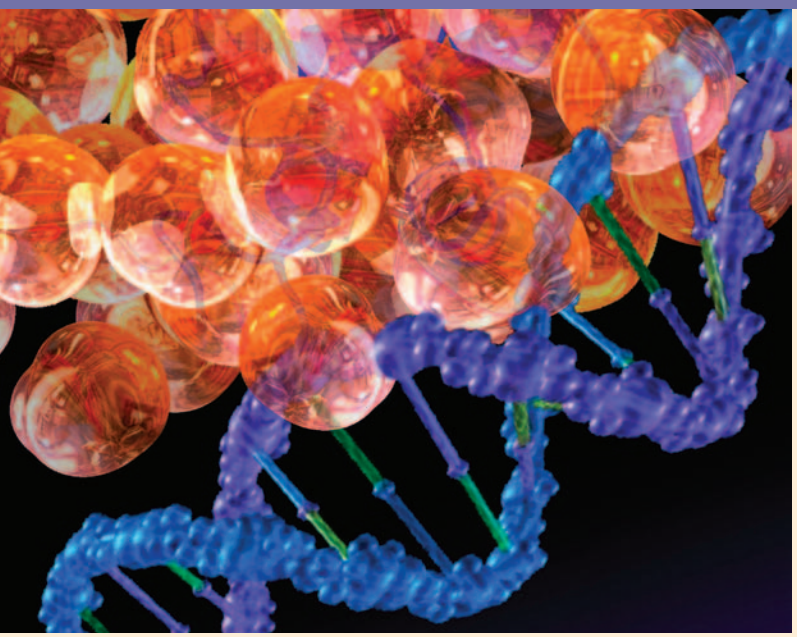
Overview of Protein:Nucleic Acid Interactions



In the late 19th century, scientists microscopically observed the association of proteins with DNA strands in the cell. Since then, researchers have shown through a variety of *in vitro* and *in vivo* assays that proteins interact with DNA and RNA to influence the structure and function of the corresponding nucleic acid. Elucidating the roles that these protein:nucleic acid complexes play in the regulation of transcription, translation, DNA replication, repair and recombination, and RNA processing and translocation continues to revolutionize our understanding of cell biology, normal cell development and mechanisms of disease.

Proteins interact with DNA and RNA through similar physical forces including electrostatic interactions (salt bridges), dipolar interactions (hydrogen bonding, H-bonds), entropic effects (hydrophobic interactions) and dispersion forces (base stacking). These forces contribute in varying degrees to proteins binding in a sequence-specific (tight) or non-sequence specific (loose) manner. Specific DNA interactions are commonly mediated by an α-helix motif in the protein which inserts itself into the major groove of the DNA, recognizing and interacting with a specific base sequence through H-bonds and salt bridges. In addition, the affinity and specificity of a particular protein:nucleic acid interaction can be increased through protein oligomerization or multi-protein complex formation (e.g., GCN4, glucocorticoid receptor, transcription initiation complexes, mRNA splicing complexes, RISC). The secondary and tertiary structure formed by nucleic acid sequences (especially in RNA) provides another important mechanism by which proteins recognize and bind particular nucleic acid sequences.

The DNA- or RNA-binding function of a protein is localized in discrete conserved domains within the protein's tertiary structure. DNA and RNA binding proteins have one or more of these modular nucleic acid binding domains linked in tandem or unlinked. An individual protein can have multiple repeats of the same nucleic acid binding domain or can have several different domains found within its structure. The identity of the individual domains is functionally important as well as their relative arrangement to each other within the protein. Several common DNA binding domains include zinc fingers, helix-turn-helix, helix-loop-helix, winged helix and leucine zipper. RNA-binding specificity and function are defined by zinc finger, KH, S1, PAZ, PUF, PIWI and RRM (RNA recognition motif) domains. Multiple domains in one protein can expand the nucleic acid recognition surface to increase specificity and affinity, organize nucleic acid topology, properly position other domains for recognition, or regulate the activity of enzymatic domains.

Proteins can bind directly to the nucleic acid or indirectly through other bound proteins, effectively creating a hierarchy of interactions. The strength of these interactions influence which assays or approaches are best for studying complex assembly. For example, transient interactions require stabilization through chemical crosslinking prior to isolation of the complexes. To understand the role these complexes play in regulating cellular processes, it is vital to learn how proteins interact with nucleic acids, to determine what proteins are present in these protein:nucleic acid complexes, and to identify the nucleic acid sequence/structure required to assemble these complexes.

Overview of Protein:Nucleic Acid Interactions

Protein:DNA Interactions

The common DNA binding domains, helix-turn-helix and zinc finger domains, are found in numerous DNA binding proteins that are co-expressed in the cell. Specificity is derived from higher order interactions involving nucleoprotein complexes. These DNA binding protein complexes find their target by "sliding" along the genome until their specific DNA docking site is discovered. Protein binding to DNA controls the structure of genomic DNA (chromatin), the transcription of RNA and DNA repair mechanisms.

A major function of protein:DNA interactions is to manage the extensive length of the genetic material contained in each cell. Chromosomes, a result of complex protein:DNA interactions, have evolved to condense this information. The chromosomal structure also plays a role in transcription. In chromosome remodeling selective portions of a chromosome unravel, making the DNA available for gene transcription, or become tightly packaged, completely silencing the transcription of the genes.

Once unraveled, the exposed DNA can be transcribed, however not all of the DNA sequence codes for proteins. The sequences between genes serve as transcriptional controls that act through proteins binding to them and include promoters, enhancers, insulators and spacers. Enhancer sequences, which can be located many kilobases away from a gene's start site, bind proteins and act as beacons to attract the transcriptional machinery. Actual transcription initiation is a two-step process involving protein:DNA interactions. First, the DNA binding domain(s) of the transcription factor proteins bind to specific DNA promoter sequences adjacent to a gene's transcriptional start site. Next, through protein:protein interactions, the transactivation domain of the transcription factor binds to and localizes the RNA Polymerase II holoenzyme, leading to initiation and production of mRNA.

Assays for Protein:DNA Interactions

A number of laboratory techniques have been developed to study these complex interactions each with a unique history, varying utility and distinct strengths and weaknesses.

DNA Electrophoretic Mobility Shift Assay (EMSA)

EMSA is used to study the binding of a protein to a known DNA oligonucleotide probe and can be used to assess the affinity of the interaction. The detection of an important interaction relies on the slower mobility of protein:DNA complexes over free DNA molecules in non-denaturing polyacrylamide or agarose gel electrophoresis. Because the rate of DNA migration is shifted or retarded upon protein binding, the assay is also referred to as a gel shift or gel retardation assay. The addition of an antibody against the target protein creates an even larger complex (antibody:protein:DNA) which migrates even more slowly. This result is known as a supershift and is used to confirm protein identity. Before EMSA was developed, protein:DNA interactions were studied primarily by nitrocellulose filter-binding assays using radioactively-labeled probes.

For more information, please see pages 13-20.

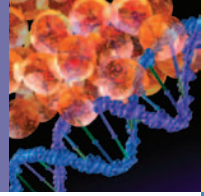
DNA EMSA

Strengths:

- detect low abundance DNA binding proteins from lysates
- test binding affinity through DNA probe mutational analysis
- non-radioactive EMSA possible using biotinylated or fluorescently-labeled DNA probes

Limitations:

- analyzes protein:DNA interactions *in vitro*
- difficult to quantitate
- need to perform supershift with antibody to confirm protein identity in a complex



Pull-down Assay

Pull-down assays are used to selectively extract a protein:DNA complex from a cell lysate. Typically, the pull-down assay uses a DNA probe labeled with a high affinity tag (e.g., biotin) which allows the probe to be recovered or immobilized. In a reaction similar to EMSA, the biotinylated DNA probe can bind with a protein in a cell lysate and then the complex is purified using agarose or magnetic beads. The proteins are then eluted from the DNA and detected by Western blot or mass spectrometry.

Alternatively, the protein may be labeled with an affinity tag or the DNA:protein complex may be isolated using an antibody against the protein of interest (similar to a supershift). In this case, the DNA interacting with the target protein is detected by Southern blot or through PCR analysis.

For more information, please see pages 38-44.

Pull-down Assay

Strengths:

- enrichment of low abundant targets
- end-labeled DNA can be generated by several methods
- isolation of intact complex
- compatible with immunoblotting and mass spectrometry analysis

Limitations:

- long DNA probes can show significant non-specific binding
- requires either Western blot or supershift quality antibodies
- nuclease-free conditions required
- assay must be performed *in vitro*

Transcription Factor Activation Assay

The transcription factor activation assay is similar to an EMSA in that both rely on the binding of a labeled DNA probe to a transcription factor. The transcription factor assay uses an ELISA-based technique to capture the activated transcription factor, followed by a chemiluminescent or colorimetric method to detect the signal. Traditional methods for measuring active transcription factors such as EMSA are cumbersome, may involve radioactivity and are not amenable to high-throughput applications.

For more information, please see pages 28-31.

Transcription Factor Assay

Strengths:

- the use of ELISA-based technology has increased speed and throughput
- amenable for drug screening
- non-radioactive

Limitations:

- not quantitative like traditional ELISAs
- data only provides relative changes in transcription factor:DNA affinity
- assay must be performed *in vitro*

Overview of Protein:Nucleic Acid Interactions

Reporter Assay

Reporter assays provide a real-time *in vivo* readout of translational activity for a promoter under study. Reporter genes consist of a target promoter DNA sequence fused to a reporter gene DNA sequence. The promoter DNA sequence is customized by the researcher; the reporter gene DNA sequence codes for a protein with detectable properties such as firefly luciferase, *Renilla* luciferase or alkaline phosphatase. When the target promoter is activated, the reporter gene enzymes are expressed. The enzyme, in turn, catalyzes a substrate to produce either light, a color change or other reaction that is detected by spectroscopic instrumentation. The signal generated from the reporter gene enzyme is an indirect determinant for the translation of endogenous proteins driven from that promoter.

Reporter Assays

Strengths:

- *in vivo* monitoring
- capture real-time data
- powerful tool for mutational analysis of promoters
- amendable to high throughput screening

Limitations:

- uses exogenous DNA
- does not address changes due to genomic sequences near the promoter of interest
- gene dosage artifacts can occur

Chromatin Immunoprecipitation (ChIP) Assay

The ChIP method can be used to monitor transcriptional regulation through histone modification (epigenetics) or transcription factor:DNA binding interactions. The ChIP method analyzes DNA:protein interactions in living cells by treating the cells with formaldehyde or other crosslinking reagents in order to stabilize the interactions for downstream purification and detection. The use of ChIP assays requires knowledge of the target protein and DNA sequence which will be analyzed because an antibody against the target protein and PCR primers for the target DNA sequence are required. The antibody selectively precipitates the protein:DNA complex from the other genomic DNA fragments and endogenous protein. The PCR primers allow specific amplification and detection of the target DNA sequence. To quantify the target DNA, quantitative PCR (qPCR) assay can be used. The ChIP method is amenable to array-based formats (ChIP on chip) or direct sequencing of the DNA captured by the immunoprecipitated protein (ChIP-Seq).

For more information, please see pages 21-27.

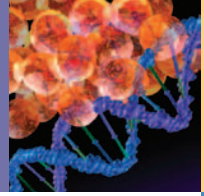
ChIP Assays

Strengths:

- capture a snapshot of specific protein:DNA interactions as they occur in living cells
- quantitative data
- ability to profile a promoter for different proteins

Limitations:

- researcher needs to source ChIP grade antibodies
- requires designing specific primers
- difficult to adapt for high throughput screening



Protein:RNA Interactions

Once an RNA transcript is produced, protein:RNA interactions occur to splice, protect, translate or degrade the message. After transcription initiation, the complementary promoter sequence in the mRNA is cleaved and the capping machinery incorporates a "GpppN" cap at the 5′ end of the mRNA. Elongation of the transcript then occurs through the recruitment of elongation factors to the message. Elongation is followed by 3′ end processing and splicing, resulting in a mature RNA transcript that is exported to the cytoplasm for translation. All of these processes require significant protein:RNA interactions and are highly regulated and complex. Many of the regulatory elements for this process lay within the regions 3′ and 5′ untranslated regions (UTRs) of the mRNA. The coding sequence for regulatory microRNAs (miRNAs) have been located in coding regions of exons, the introns and exons of non-coding genes and also in repetitive elements. An increased emphasis has been placed on the significance of the non-coding RNAs and their roles in cellular regulation and disease states. However, tools for the study of critical protein:RNA interactions have been limited.

The UTR regions of mRNA contain sequence elements that recruit RNA-binding proteins for post-transcriptional regulation and protein translation. In addition, these elements promote transcript stability or degradation and can direct subcellular localization of the RNA. These RNA regulatory elements vary in length, but rely on both primary and secondary structure for RNA:protein binding interactions. For example, iron regulation in the cell is a tightly regulated process where a protein:RNA interaction is key to maintaining iron homeostasis. Target genes, such as the iron storage protein ferritin or the transferrin receptor, contain a small (~28 nucleotides) consensus iron responsive element (IRE) in their respective 5′ or 3′ UTR. The iron responsive protein (IRP) responds to cellular iron status by binding the IRE element. Under iron-starved conditions, IRP remains bound to the IRE element to suppress translation of iron storage proteins. Under iron-rich conditions, IRE binding activity of IRP is lost and iron storage proteins are translated. Many of these RNA consensus elements have been identified and classified into different families based on sequence and function. The 3′ UTR also contains recognition elements for miRNA which are responsible for repression of protein translation of target mRNA.

miRNAs are ubiquitous and comprise a large class of non-coding RNAs that initiate post-transcriptional silencing of target mRNA. Over 700 miRNAs have been identified in the human genome (www.mirbase.org). These miRNA have binding recognition sequences in 57.8% of human mRNAs, with 72% containing of those mRNAs having multiple miRNA recognition sites. miRNA begins as a 70-100 nucleotide transcribed RNA (*pre-miRNA*) containing a 6-8 nucleotide seed region at the 5′ end for mRNA binding. The miRNA is then cleaved by DROSHA, a nuclear endoribonuclease III. The pre-miRNA then associates with double-stranded RNA-binding proteins and is actively exported to the cytoplasm, a process dependent on Exportin 5 and Ran GTPase. The *pre-miRNA* is then further processed in an RNP complex consisting of Argonaute proteins and Dicer (endoribonuclease III) into the mature 19-22 nucleotide *miRNA*. The miRNA:Argonaute complex then binds to target genes and recruits additional unidentified proteins for regulation of target genes.

In most cases, the mRNA is repressed through mRNA degradation, deadenylation or storage in cytoplasmic mRNA processing bodies (P-bodies), although in some instances mRNA may also be upregulated. Several models of mRNA repression and degradation have been proposed, but none has been adopted. MicroRNA research is rapidly expanding and key protein:RNA interactions are being investigated to further understand the role of miRNA in cell growth, differentiation and carcinogenesis.

Overview of Protein:Nucleic Acid Interactions

Assays to Study Protein:RNA Interactions

Assays for identification of protein:RNA interactions are quite tedious. Both the RNA and protein must fold correctly for proper binding and caution must be taken not to introduce additional proteases and nucleases into the reaction. Protein:RNA interactions have been identified utilizing EMSA, pull-down assays, FISH/ISH co-localization studies, Northwestern analysis and RNase protection assays. For miRNA, miRNA labeling techniques are available, as well as procedures for enrichment of miRNA. The most common techniques for detection of miRNA:RNA or protein interactions is through qPCR and microarrays. Most of these assays consist of a labeling reaction for detection and a binding reaction. FISH/ISH co-localization studies use a labeled RNA in conjunction with antibody staining for the protein or a fluorescent protein construct. Several assays will be discussed below, including their limitations.

RNA Electrophoretic Mobility Shift Assay (RNA EMSA)

An RNA EMSA is an *in vitro* technique which detects protein:RNA interactions through changes in gel electrophoresis migration patterns. A labeled RNA probe is incubated with a protein sample (typically from a cell lysate) to initiate binding. The binding reaction is then separated via non-denaturing polyacrylimide gel electrophoresis. Like DNA:protein complexes, a RNA:protein complex migrates more slowly than a free RNA probe in the gel causing a change in migration pattern, or a shift, of the detectable RNA probe which indicates a complex has been formed. Specificity is determined through a competition reaction in which excess unlabeled RNA is added to the binding reaction, resulting in a decrease in the shifted signal. Alternatively, the protein:RNA complex may be cross-linked and the reaction run on a denaturing gel. Specificity is determined through visualization of a single shifted band that can be competed away with an unlabeled probe. RNA probes are usually labeled radioactively, although fluorescent and chemiluminescent detection is possible. Non-radioactive RNA end-labeling techniques are limited, but both biotin and fluorescent labeling techniques have been recently introduced.

For more information, please see pages 13-20.

RNA EMSA

Strengths:

- non-radioactive detection available
- easy to screen RNA mutants for binding efficiency
- compatible with RNA labeled via run-off transcription reactions, but best results from end-labeled RNA probes

Limitations:

- homebrew experiments require significant reagent optimization for success
- lengthy protocol including native electrophoresis step
- antibodies needed to determine identity of RNA binding protein
- requires labeled RNA probe design and synthesis

RNA Pull-down Assay

RNA pull-down assays selectively extract a protein:RNA complex from a sample. Typically, the RNA pull-down assay takes advantage of high affinity tags, such as biotin or azido-phosphine chemistry. An RNA probe can be biotinylated, complexed with a protein from a cell lysate and then purified using agarose or magnetic beads. Alternatively, the protein may be labeled, or the RNA:protein complex may be isolated using an antibody against the target protein. The RNA is then detected by Northern blot or through RT-PCR analysis and proteins are detected by Western blotting or mass spectrometry.

For more information, please see pages 38-44.

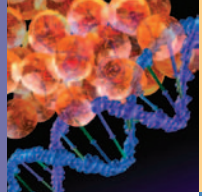
RNA Pull-down Assay

Strengths:

- enrichment of low abundant targets
- isolation of intact complex
- compatible with immunoblotting and mass spectrometry analysis

Limitations:

- RNA secondary structure is important for function, so end-labeling is preferred
- inefficient elution from the resin
- nuclease-free conditions required



Fluorescent *In Situ* Hybridiation Co-localization

Fluorescent *in situ* hybridization (FISH/ISH) techniques require detection of both an RNA transcript and a protein of interest using RNA probes and antibodies. FISH/ISH detects the position and abundance of an RNA and protein in a cell or tissue sample. The readout is visual (usually imaged via microscopy) and a co-localized signal for both the target RNA and protein indicates possible complex formation. A labeled RNA probe must be generated for detection of a particular sequence of RNA and the protein may be detected using antibody staining or fluorescent protein constructs.

FISH/ISH Technique

Strengths:

- provides a snapshot of RNA:protein interactions as they occur *in vivo*
- possible to multiplex with different substrates
- delivers publication quality images

Limitations:

- tedious procedure, requiring denaturation, hybridization and detection
- detection usually requires signal amplification.
- cells/tissue must be preserved
- quantitative software is necessary to determine co-localization

DNA:Protein Interactions References

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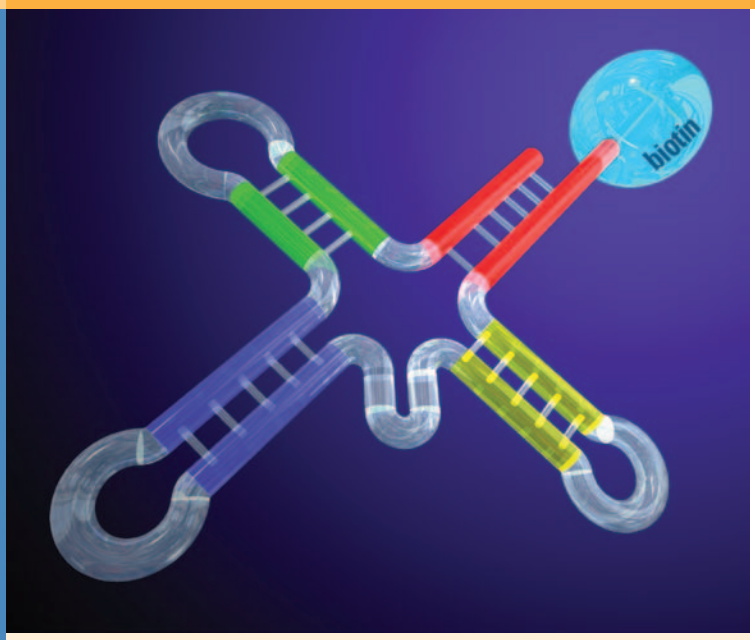
EMSA Probe Labeling Systems



An Electrophoretic Mobility Shift Assay (EMSA) resolves the difference in size between a nucleic acid probe bound to a protein complex and an unbound probe. In order to detect the nucleic acid probe in either the bound or free-state, labels are incorporated into the probe. An ideal label is small and located at a terminus of the nucleic acid probe to prevent steric interference with the protein-nucleic acid complex. Additionally, a label must be stable enough to retain signal throughout the EMSA procedure, which includes *in vitro* incubation, native polyacrylamide gel electrophoresis (PAGE), transfer to a positively charged nylon membrane, crosslinking with ultraviolet light and final detection.

Common EMSA nucleic acid probe labels include radioactive phosphates, biotin or fluorophores. Radioactive labels are visualized by autoradiography film and provide high sensitivity. Biotin labels detected with streptavidin horseradish peroxidase (HRP) can be coupled with highly sensitive chemiluminescent substrates to provide equivalent sensitivity to radioactive methods without extended exposure to X-ray film. Fluorescent probes are visualized using gel imaging equipment possessing the proper excitation energy source and the proper filter sets but typically provide less sensitivity.

Optimal nucleic acid probe labeling for EMSA utilizes an enzymatic method that targets a terminus. Alternatively, enzymatic methods which incorporate labels throughout the probe sequence can also be used, however they yield internal labels which may affect protein-nucleic acid binding sites. Lastly, chemical crosslinkers that target nucleic acid functional groups can be used to label nucleic acid probes.

Enzymatic Methods for Probe End Labeling

EMSA probe labels are typically incorporated by enzymatic methods, but can also be attached to nucleic acid strands during commercial synthesis. The limitation of commercially prepared and labeled probes is high cost in contrast to enzymatic labeling performed in house. Common enzymatic labeling procedures target either the 3′-end or 5′-end of the EMSA probe, which eliminate steric hindrance.

DNA 3′-end Biotinylation

DNA probe biotinylation can be performed using terminal deoxynucleotidyl transferase (TdT) and biotinylated ribonucleotides, such as Biotin-11-UTP (see page 11). TdT is a template-independent polymerase which typically adds numerous deoxynucleotides to the 3′ terminus of a DNA strand but can be held to 1-3 incorporation events by when using ribonucleotides. Because TdT is template independent, the sequence of the DNA probe will not affect labeling efficiency significantly, however the structure of the DNA probe does influence labeling efficiency. TdT has an affinity for single-stranded DNA 3′ ends, but will work with double-stranded DNA that possesses a 3′ overhang. Blunt end or 5′ overhang double-stranded DNA probes exhibit the lowest affinity for TdT. Common sources for DNA probe templates include unlabeled single-stranded synthetic primers or double-stranded DNA linear fragments extracted from circular plasmids using restriction endonucleases which create 3′ overhangs (“sticky ends”). Unlabeled single-stranded synthetic primers require labeling of one or both strands individually and then annealing to form a double-stranded probe. Annealing can be performed quickly by mixing equimolar amounts of each strand, heating to 95°C for 10 minutes and cooling to room temperature. Double-stranded DNA probes can be used in an EMSA immediately following biotinylation. Quantitation of labeling efficiency is typically performed by dot-blotting against a control biotinylated DNA probe.

RNA 3′-end Biotinylation

RNA 3′-end terminus labeling requires T4 RNA ligase to incorporate a biotinylated nucleotide. RNA ligation using T4 RNA ligase requires a 3′-OH (from the desired RNA), and a 3′,5′ nucleotide (bis)phosphate. The Thermo Scientific Pierce 3′ RNA Labeling Kit contains T4 RNA ligase and a cytidine (bis)phosphate nucleotide with a biotinylated linker for detection (Product # 20160, see page 11). T4 RNA ligase typically labels with a 70-80% efficiency in reactions containing 50 pmol RNA probes, incubated for two hours at 16°C; see page 11. Although RNA is single-stranded, many probes possess secondary structures such as hairpins, which are a function of RNA probe length and sequence. Significant secondary structure can negatively affect probe labeling by T4 RNA ligase, however methods to relax RNA secondary structure exist such as heating the RNA prior to labeling, the addition of DMSO to the labeling mixture, extended incubations with enzyme (12 hours at 16°C) and increased biotinylated nucleotide concentration. Similar to DNA biotinylation, RNA biotinylation efficiency can also be determined using dot-blotting and spot densitometry of a labeled RNA sample compared to a biotinylated RNA control.

Nucleic acid probe 5´-end radiolabeling with ^{32}P

Traditional EMSAs have used radiolabeled nucleic acid probes to detect gel shifts. Radiolabeled probes are generated using radioactive ATP (wherein the gamma phosphate is ^{32}P-labeled) and T4 polynucleotide kinase (PNK). PNK transfers the gamma phosphate from radiolabeled ATP to the 5´ hydroxyl terminus of a DNA or RNA probe. The PNK transfer is slightly more efficient with a single-stranded overhang or a blunt end fragment. PNK can even add phosphates to mononucleotides. Advantages of ^{32}P labeling include high sensitivity and no steric hindrance to potential protein-nucleic acid complex formation, since there is no detectable difference in structure using a radiolabel. While still popular, radioactive methods have significant disadvantages with respect to safety, signal intensity and probe stability. Alternative labeling using biotinylated nucleotides produce safe and stable nucleic acid probes.

Enzymatic Methods for Internal Probe Labeling

Nick translation methods for internal DNA probe biotinylation exist, but have not been tested with DNA EMSAs. For an RNA gel shift assay, *in vitro* run-off transcription reactions can be used to generate labeled RNA probes. However the method is not optimal as internal labels pose a threat to RNA secondary structure or unstable protein-RNA complexes. Optimization is required to titrate the amount of biotinylated nucleotide used to prevent over-labeling. Additionally, internal biotins may be masked by proteins binding the RNA probe, limiting access to avidin-based detection reagents.

RNA biotinyation with *in vitro* run-off transcription

RNA run-off transcription is a quick way to generate RNA probes from a DNA template using biotinylated nucleotides. Typically, one of the four nucleotides is biotinylated and titrated to a concentration which does not result in over-labeling. Different RNA polymerases derived from bacteriophages can be used to generate RNA probes using a DNA sequence as a template. Three common RNA polymerases derived from bacteriophage are used, T7, SP6 and T3 RNA polymerases. Bacteriophage RNA polymerases are used because of their simplified structure, in contrast to the multiple subunit RNA polymerase found in higher order mammals. The choice of which RNA polymerase to use is dependent on the promoter sequences found on the vector containing the target DNA template. Each promoter element recruits a specific RNA polymerase. A particular promoter element can be added to a DNA sequence of interest using PCR.

- T7 RNA polymerase
  (promoter sequence: 5′ TAATACGACTCACTATAGGG 3′)
- SP6 RNA polymerase
  (promoter sequence: 5′ AATTTAGGTGACACTATAGAA 3′)
- T3 RNA polymerase
  (promoter sequence: 5′ AATTAACCCTCACTAAAGGG 3′)

Run-off transcription is quick, and numerous commercial kits are available which provide a particular RNA polymerase and transcription reaction buffer. A labeled nucleotide is often available separately. Additionally, run-off transcription can yield high quantities of RNA probe which can be stored in aliquots at -80°C for future experiments.

Chemical Methods for Nucleic Acid Probe Labeling

Chemical biotinylation of nucleic acid probes is possible using reagents which possess both the biotin heterocyclic ring structure and a reactive chemical group that is optimized for bioconjugation to functional groups common found in biomolecules. For chemical nucleic acid probe labeling reagents, see page 32.

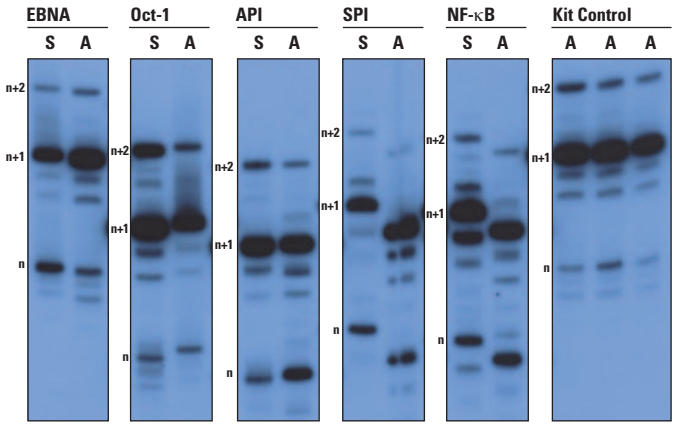
DNA 3′ End Biotinylation Kit

A complete kit for labeling the 3′ end of DNA with biotin.

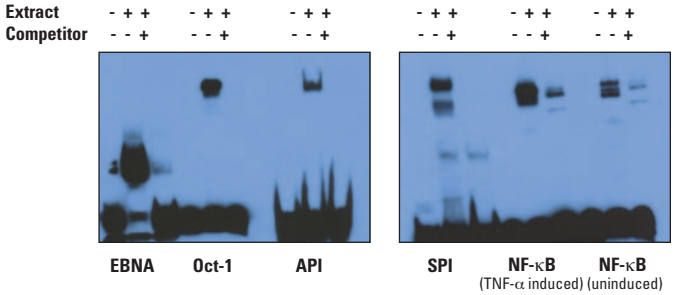
The Thermo Scientific Biotin 3′ End DNA Labeling Kit uses terminal deoxynucleotidyl transferase (TdT) to catalyze nontemplate-directed nucleotide incorporation onto the 3′-OH end of DNA.[1,2] TdT exhibits a substrate preference of single-stranded DNA, but it will label duplex DNA with 3′ overhangs and blunt duplexes, albeit with a lower efficiency.[3] The Biotin 3′ End DNA Labeling Kit has been optimized to incorporate 1-3 biotinylated ribonucleotides (biotin-11-UTP) onto the 3′ end of DNA strands. This labeling strategy has the advantage of localizing the biotin to the 3′ end of the probe where it will be less likely to interfere with hybridization or sequence-specific binding of proteins. Biotin-labeled DNA probes can be used to facilitate non-isotopic detection in a variety of applications including electrophoretic mobility shift assays (EMSA), Northern or Southern blots, colony hybridizations or *in situ* hybridizations.

Highlights:

- Non-isotopic labeling eliminates the hassle of hazardous radioactive materials or difficult-to-dispose-of waste
- 1-3 biotinylated ribonucleotides onto the 3′ end of DNA strands for less interference with hybridization or sequence-specific binding of proteins
- Biotin-labeled probes are stable for more than one year
- 30-minute labeling procedure is fast and efficient



Sequencing gel analysis of labeling efficiency. Ten different oligos (ranging in size from 21-25 nt) were labeled using the Thermo Scientific Biotin 3′ End DNA Labeling Kit. The products from the TdT reaction were then radiolabeled using T4 polynucleotide kinase (PNK) and [γ-^{32}P]ATP. The PNK reactions were run on a 20% acrylamide/8 M urea/TBE. The position of the starting oligo (no biotin) is denoted by "n." Incorporation of biotin-labeled ribonucleotide by TdT is limited to one or two incorporations per strand (positions "n+1" and "n+2," respectively). Labeling efficiencies ranged from 72% (EBNA sense strand) to 94% (Oct-1 sense strand). The kit control oligo labeled with 88-94% efficiency.



EMSA results using 3′ biotin-labeled DNA duplexes. The sense and antisense strands were labeled using the Thermo Scientific Biotin 3′ End DNA Labeling Kit and hybridized for 4 hours at room temperature to form duplexes containing the binding sites for the indicated transcription factors. Gel shift assays were performed using the Thermo Scientific LightShift Chemiluminescent EMSA Kit using 20 fmol duplex per binding reaction. The source of the transcription factors was a HeLa nuclear extract prepared using Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Reagents (Product # 78833) (2 µl or 6-7 µg protein per reaction). In the case of the NF-κB system, nuclear extracts were made from HeLa cells that had been induced with TNFα or cells that were untreated. Competition reactions containing a 200-fold molar excess of unlabeled duplex were performed to illustrate the specificity of the protein:DNA interactions.

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Ordering Information

Product #	Description	Pkg. Size
89818	**Biotin 3′ End DNA Labeling Kit**	Kit
	Sufficient components for 20 labeling reactions.	
	Includes: 5X TdT reaction buffer	1 ml
	Terminal deoxynucleotidyl transferase (TdT)	50 µl
	Biotin-11-UTP	100 µl
	Unlabeled control oligo	140 µl
	Biotin-control oligo	40 µl

RNA 3′ End Biotinylation Kit

A non-radioactive, non-interfering RNA labeling method.

Regulation of cellular function is dependent on critical RNA interactions with proteins and other RNA, including miRNA. These interactions have been difficult to isolate and highly dependent on maintaining RNA secondary structure. To enrich for these interactions it is often necessary to label the RNA. The Thermo Scientific Pierce RNA 3′ End Biotinylation Kit enables rapid non-radioactive RNA labeling with minimal interference to the RNA secondary structure.

Highlights:

- **Non-radioactive** – incorporates a biotin label with detection sensitivity comparable to radioactivity
- **Fast** – RNA can be labeled in 0.5-2 hours with minimal downstream processing
- **Easy to use** – RNA ligase and optimized reaction buffer are included
- **Single label** – results in minimal disturbance of RNA secondary structure
- **Flexible** – labels synthetic and *in vitro* transcribed RNA with 22–450 nucleotides

The Pierce® RNA 3′ Biotinylation Kit contains T4 RNA ligase to attach a single biotinylated nucleotide to the 3′ terminus of an RNA strand. The unique feature of this kit is the biotinylated cytidine (bis)phosphate, which contains a 3′, 5′ phosphate on the ribose ring to accommodate the ligation reaction and a biotin on the cytidine for detection (Figure 1). The kit also contains a non-labeled RNA strand as a positive control and a biotinylated-RNA probe to quantitate labeling efficiency. To enhance biotinylation efficiency and RNA stability, RNase inhibitor, glycogen and ligation-enhancing reagents are included.

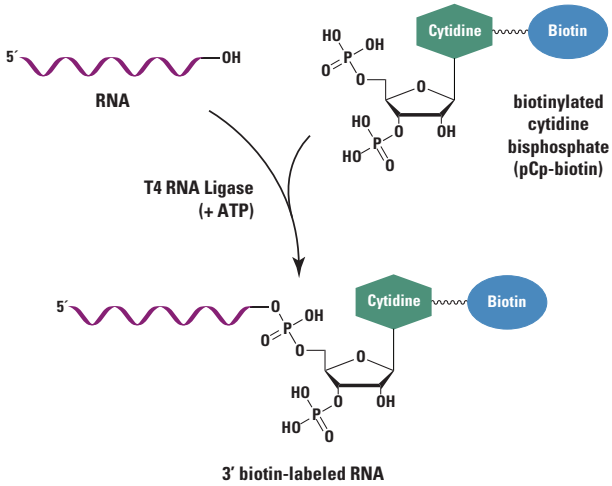

Figure 1. Scheme of T4 RNA ligation reaction. RNA ligation using T4 RNA ligase requires a 3′-OH (from the desired RNA), and a 3′, 5′ nucleotide (bis)phosphate. The Thermo Scientific Pierce 3′ RNA Labeling Kit contains T4 RNA ligase and a cytidine (bis)phosphate nucleotide with a biotinylated linker for detection.

The kit provides a starting point for the ligation reaction. Typically, the reaction requires 50 pmol of RNA is ligated with a 20-fold excess of biotinylated nucleotide for 2 hours at 16°C; however, short RNAs with a minimally complicated secondary structure can be ligated in 30 minutes at 37°C (e.g., RNA polymerase RNA template). Large or structurally complex RNAs require more incubation time (e.g., Let-7 and hTR) (Table 1). Further optimization is achieved by altering the ligation ratio, increasing the incubation time, or using DMSO to relax RNA structure. Once biotinylated, the labeled RNA is easily precipitated to remove reaction byproducts. The probe can then be used in downstream applications such as RNA electrophoretic mobility shift assays (LightShift Chemiluminescent RNA EMSA, Product # 20158), RNA pull-down assays and miRNA profiling.

Table 1. Labeling efficiency of different RNA sources and lengths.

RNA	Type	RNA source	Length (bp)	Efficiency (%)§	Method Notes	Reference
IRE (iron-responsive element)	3′ or 5′ UTR element	Synthetic	28	76	2 hours at 16°C	Leibold, *et al.*
RNA polymerase template	RNA	Synthetic	42	80†	2 hours at 16°C	McKinley, *et al.*
Mir-16-1 miRNA	Mature microRNA	Synthetic	22	70	Overnight at 16°C	www.mirbase.org
TNF ARE	3′ UTR element	Synthetic	37	77	2 hours at 16°C	Hall-Pogar, *et al.*
Let-7 pre-miRNA	Pre-micro RNA	*in vitro* transcribed	~70	70	Overnight at 16°C	Piskounova, *et al.*
hTR (telomerase RNA)	Catalytic RNA	*in vitro* transcribed	350	74	Overnight at 16°C	O'Connor, *et al.*

§ *Ligation efficiency was determined by densitometry analysis on dot blots from three separate ligation reactions using serial dilutions of probe. Synthetic biotinylated RNA was used as the control, and concentrations were normalized.*

† *RNA polymerase template RNA may be ligated at 37°C for 30 minutes with > 80% efficiency.*

To monitor the effectiveness of RNA biotinylation, the control RNA labeling efficiency was assessed by two methods, dot blotting with the Thermo Scientific Chemiluminescent Nucleic Acid Detection Module (Product # 89880) or spectroscopically with the Thermo Scientific Fluorescent Biotin Quantitation Kit (Product # 46610). Biotin end-labeled IRE and RNA polymerase template RNAs have comparable sensitivity to a synthetically biotinylated RNA (Figure 2). The 50 pmol reaction provided more than enough RNA to perform an EMSA, and both RNAs could be diluted at least 25- to 50-fold (5-10 nM).

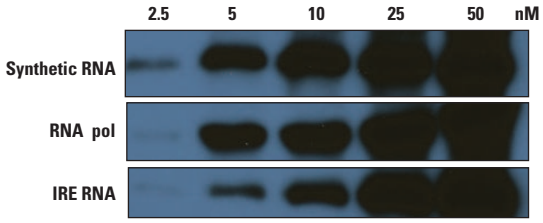

Figure 2. End-labeled RNAs have similar sensitivity to synthetically labeled RNA. Two end-labeled RNA probes made with the Pierce 3′ End Biotinylation Kit (RNA pol and IRE RNA) and a synthetic biotinylated RNA probe (Synthetic RNA) were electrophoresed on a 10% acrylamide/8M urea gel, transferred to a nylon membrane, UV crosslinked, and detected using the Thermo Scientific Chemiluminescent Detection Module (Product # 89880). RNA probe concentrations are indicated (nM). The membranes were exposed to film for 5 seconds.

RNA lengths vary from 18 to 22 nucleotides (miRNA) to hundreds of bases with a variety of secondary structures. RNA probes were derived synthetically as well as transcribed *in vitro* using T7 RNA polymerase. The different RNA used in this study had diverse secondary structure and lengths. Each of the four synthetic and both *in vitro* transcribed RNAs had ligation efficiencies > 70%, demonstrating that the kit is flexible and efficient biotinylation for RNA of different length, secondary structure, and source (Table 1).

Once labeled, a biotinylated RNA probe can be used as bait to study RNA interactions with other molecules. The biotin group enables purification and detection using biotin-binding proteins immobilized on a solid support or conjugated to enzymes such as horseradish peroxidase (HRP). To demonstrate functionality, the biotinylated RNA probes were incubated with extract containing a RNA binding protein of interest and processed with the Thermo Scientific LightShift Chemiluminescent RNA EMSA Kit (Product # 20158). Biotinylated IRE, RNA polymerase template, and Let-7 pre-miRNA probes functionally bound their respective RNA binding proteins, IRP, RNA polymerase, and Lin28 (Figure 3). RNA 3′ end biotinylation produces a stable probe that is effective for a longer time period than radiolabeled probes. Furthermore, end-labeling minimally disrupts secondary structure, can reduce background, and is much less expensive compared to synthetically labeled RNA.

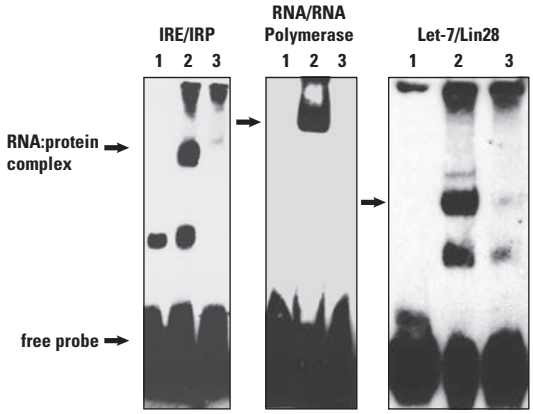

Figure 3. Biotinylated end-labeled RNA functionally binds RNA binding proteins. Probes (5-10 nM) were incubated with 4-5 µg of cell extract in the binding reaction. EMSA was performed using reagents from the LightShift® Chemiluminescent RNA EMSA Kit (Product # 20158). The lower band labeled "free probe" represents unbound biotinylated RNA probe. The higher molecular weight band, or shifted band, represents an RNA:protein complex. **Exposure times**: IRE-IRP – 2 minutes; RNA-RNA polymerase – 1 minute; Let-7-Lin28 – 5 minutes. **Lane 1:** Free probe; **Lane 2:** Control binding reaction; **Lane 3:** Control binding reaction plus 100-fold excess of unlabeled probe.

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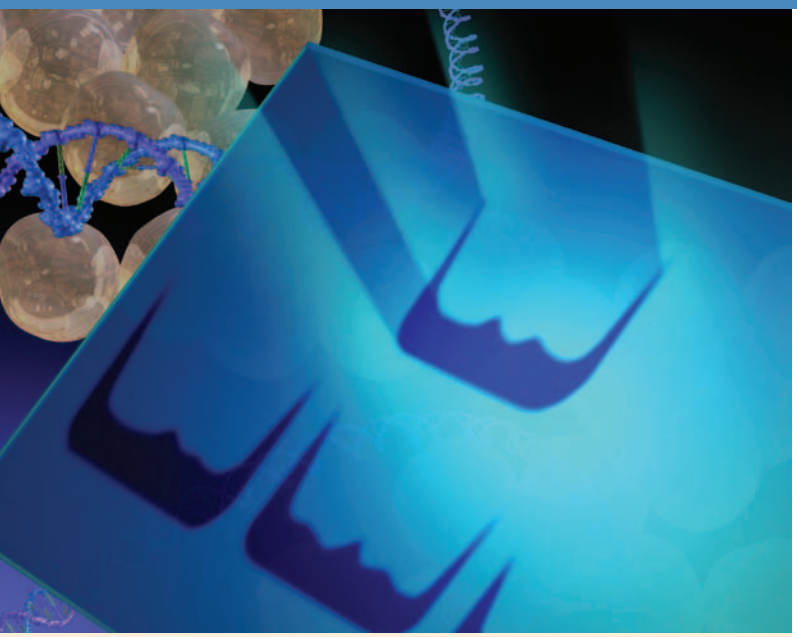
www.mirbase.org

Ordering Information

Product #	Description	Pkg. Size
20160	Pierce RNA 3′ End Biotinylation Kit	Kit

Electrophoretic Mobility Shift Assays (EMSA)



Introduction to the EMSA (Gel-shift) Technique

The interaction of proteins with DNA is central to the control of many cellular processes including DNA replication, recombination and repair, transcription and viral assembly. One technique that is central to studying gene regulation and determining protein:DNA interactions is the electrophoretic-mobility shift assay (EMSA).

The EMSA technique relies on the fact that protein:DNA complexes migrate more slowly than free DNA molecules when subjected to non-denaturing polyacrylamide or agarose gel electrophoresis.[1,2] Because the rate of DNA migration is shifted or retarded upon protein binding, the assay is also referred to as a gel-shift or gel-retardation assay. Until conception of the EMSA by Fried and Crothers[3] and Garner and Revzin,[4] protein:DNA interactions were studied primarily by nitrocellulose filter-binding assays.[5]

An advantage of studying DNA:protein interactions by an electrophoretic assay is the ability to resolve complexes of different stoichiometry or conformation. Another major advantage for many applications is that the source of the DNA-binding protein may be a crude nuclear or whole cell extract rather than a purified preparation. Gel-shift assays can be used qualitatively to identify sequence-specific DNA-binding proteins (such as transcription factors) in crude lysates and, in conjunction with mutagenesis, to identify the important binding sequences within a given gene's upstream regulatory region. EMSAs can also be used quantitatively to measure thermodynamic and kinetic parameters.[3,4,6,7]

The ability to resolve protein:DNA complexes depends largely upon the stability of the complex during the brief time (approximately one minute) it is migrating into the gel. Sequence-specific interactions are transient and are stabilized by the relatively low ionic strength of the electrophoresis buffer used. Upon entry into the gel, protein complexes are quickly resolved from free DNA, in effect freezing the equilibrium between bound and free DNA. In the gel, the complex may be stabilized by "caging" effects of the gel matrix, meaning that if the complex dissociates, its localized concentration remains high, promoting prompt reassociation.[3,6] Therefore, even labile complexes can often be resolved by this method.

Critical EMSA Reaction Parameters

Nucleic Acid Probe

Typically, linear DNA fragments containing the binding sequence(s) of interest are used in EMSAs. If the target DNA is short (20-50 bp) and well defined, complementary oligonucleotides bearing the specific sequence can be synthesized, purified by gel or HPLC, and annealed to form a duplex. Often, a protein:DNA interaction involves the formation of a multiprotein complex requiring multiple protein binding sequences. In this situation, longer DNA fragments are used to accommodate assembly of multiprotein complexes. If the sequence is larger (100-500 bp), the DNA source is usually a restriction fragment or PCR product obtained from a plasmid containing the cloned target sequence. Protein:DNA complexes formed on linear DNA fragments result in the characteristic retarded mobility in the gel. However, if circular DNA is used (e.g., minicircles of 200-400 bp), the protein:DNA complex may actually migrate faster than the free DNA. Gel-shift assays are also good for resolving altered or bent DNA conformations that result from the binding of certain protein factors.

Gel-shift assays need not be limited to DNA:protein interactions. RNA:protein interactions[8,9] as well as peptide:protein interactions[10] have also been studied using the same electrophoretic principle. RNA secondary structure is important for RNA:protein complexes. Steric hindrance due to the probe label should be prevented to maintain this secondary structure. RNA probes can be biotinylated at the 3′ end (Product # 20160, page 11) to help avoid steric hindrance issues. Fully optimized RNA EMSA Kits are also available (Product # 20158, page 18).

If large quantities of DNA are used in EMSA reactions, the DNA bands can be visualized by ethidium bromide staining. However, it is usually preferable to use low concentrations of DNA, requiring the DNA to be labeled before performing the experiment. Traditionally, DNA is radiolabeled with ^{32}P by incorporating an [α-^{32}P]dNTP during a 3′ fill-in reaction using Klenow fragment or by 5′ end labeling using [γ-^{32}P]ATP and T4 polynucleotide kinase. Non-radioactive methods are available to label and detect nucleic acid probes with biotin.

Nonspecific Competitor

Nonspecific competitor DNA such as poly(dI•dC) or poly(dA•dT) is included in the binding reaction to minimize the binding of nonspecific proteins to the labeled target DNA. For RNA EMSAs, tRNA is used to block non-specific binding interactions. These repetitive polymers provide nonspecific sites to adsorb proteins that will bind to any general nucleic acid sequence. To maximize effectiveness, the competitor DNA probe must be added to the reaction along with the extract prior to the labeled DNA target. Besides poly(dI•dC) or other nonspecific competitor nucleic acid, a specific unlabeled competitor sequence can be added to the binding reaction. A 200-fold molar excess of unlabeled target is usually sufficient to out-compete any specific interactions. Thus, any detectable specific shift should be eliminated by the presence of excess unlabeled specific competitor (Figure 4). The addition of a mutant or unrelated sequence containing a low-affinity binding site, like poly(dI•dC), will not compete with the labeled target and the shifted band will be preserved.

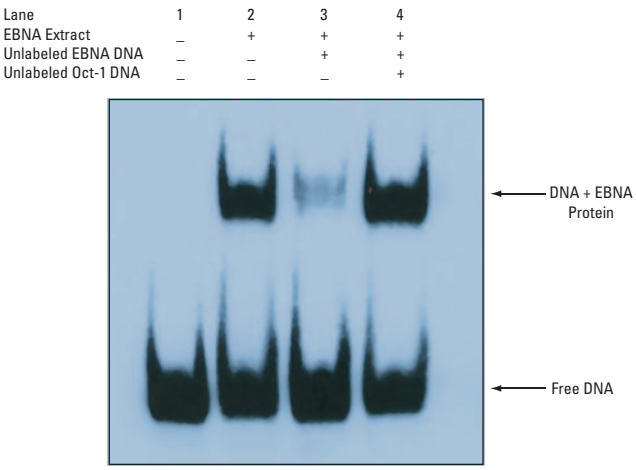


Figure 4. EMSA results using the EBNA control system. Biotin-labeled 60 bp duplex bearing the EBNA-1 binding sequence was incubated with an extract in which the EBNA-1 protein was overexpressed. The binding buffer was supplemented with 50 ng/µl poly(dI•dC), 10% glycerol and 0.05% NP-40. Exposure time was 30 seconds with X-ray film.

Binding Reaction Components

Factors that affect the strength and specificity of the protein:DNA interactions under study include the ionic strength and pH of the binding buffer; the presence of nonionic detergents, glycerol or carrier proteins (e.g., BSA); the presence/absence of divalent cations (e.g., Mg^{2+} or Zn^{2+}); the concentration and type of competitor DNA present; and the temperature and time of the binding reaction. If a particular ion, pH or other molecule is critical to complex formation in the binding reaction, it is often included in the electrophoresis buffer to stabilize the interaction.

Gel Electrophoresis

Non-denaturing TBE-polyacrylamide gels or TAE-agarose gels are used to resolve protein:DNA complexes from free DNA. The gel percentage required depends on the size of the target DNA and the size, number and charge of the protein(s) that bind to it. Polyacrylamide gels in the range of 4-8% are typically used, although it is not uncommon for higher percentage gels to be used with certain systems. Agarose gels (0.7-1.2%) can be used to resolve very large complexes, such as *E. coli* RNA polymerase (~460 kDa).

Gels are pre-run at a constant voltage until the current no longer varies with time. The primary reasons for pre-running gels are to remove ammonium persulfate to distribute/equilibrate any stabilizing factors or ions that were added to the electrophoresis buffer, and to ensure a constant gel temperature.

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EMSA Applications

The Supershift Reaction

This technique can aid in the identification of the DNA-bound protein. This is accomplished by including an antibody, specific for the DNA-binding protein, to the binding reaction. If the protein of interest binds to the target DNA, the antibody will bind to that protein:DNA complex, further decreasing its mobility relative to unbound DNA in what is called a "supershift." In addition to antibodies, supershift reactions could include other secondary or indirectly bound proteins.

Shift-Western Blot

This application involves transferring the resolved protein:DNA complexes to stacked nitrocellulose and anion-exchange membranes. Proteins captured on the nitrocellulose membrane can be probed with a specific antibody (Western blot) while autoradiography or chemiluminescent techniques can detect the DNA on the anion-exchange membrane.

Alternatively, DNA can be labeled with a biotinylated or hapten-labeled dNTP, then probed and detected using an appropriately sensitive fluorescent or chemiluminescent substrate. We offer a chemiluminescent EMSA system (Product # 20148) and a kit to facilitate labeling DNA with biotin (Product # 89818). The EMSA Kit offer detection levels rivaling that of isotopic-based systems.

NE-PER Nuclear and Cytoplasmic Protein Extraction Reagent Kit

Nuclear and cytoplasmic protein extraction from cultured cells and tissue for Electrophoretic Mobility Shift Assay (EMSA).

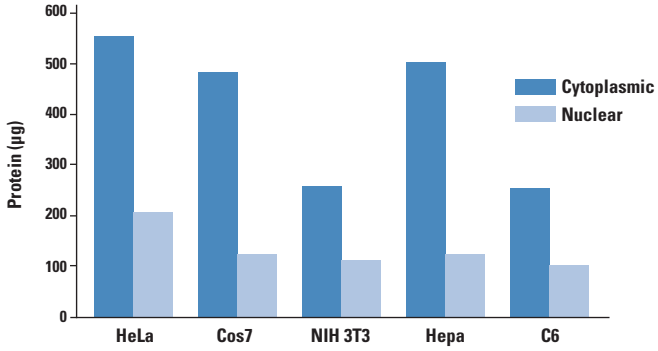
The Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Kit provides for efficient cell lysis and extraction of separate cytoplasmic and nuclear protein fractions in less than two hours. The nuclear protein extraction method involves simple, stepwise lysis of cells and centrifugal isolation of nuclear and cytoplasmic protein fractions. A benchtop microcentrifuge, tubes and pipettors are the only tools required. The NE-PER® Reagents efficiently solubilize and separate cytoplasmic and nuclear proteins into fractions with minimal cross-contamination or interference from genomic DNA and mRNA. The isolated proteins can be used to perform immunoassays as well as protein interaction studies such as mobility shift assays (EMSA) and pull-down assays. Isolating cytosolic or nuclear protein fractions enriches for target proteins and eliminates non-specific proteins which increase background. For a DNA EMSA experiment, typically 3-6 µg of nuclear protein is used in each binding reaction. For an RNA EMSA, 1-10 µg of cytosolic protein or nuclear protein is used in a binding experiment. The amount of lysate used is dependent on the abundance of the targeted protein.

Highlights:

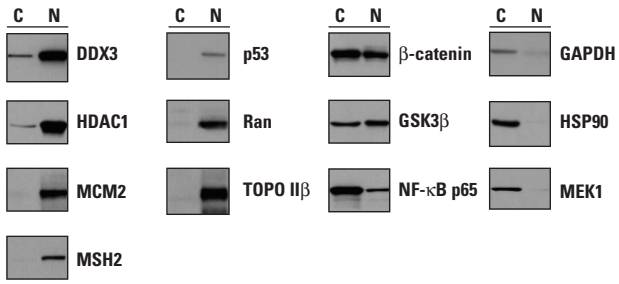
- **Fast** – obtain nuclear and cytoplasmic fractions in less than two hours
- **Proven** – the NE-PER Reagent Kit is referenced in more than 950 distinct publications
- **Scalable** – two kit sizes for producing extracts from cells and tissues
- **Convenient** – simple instructions do not require ultracentrifugation over gradients
- **Compatible** – use for downstream assays, including Western blotting, gel-shift assays, protein assays, reporter gene assays and enzyme activity assays

There are a variety of methods available to isolate nuclei and prepare nuclear protein extracts. Most of the methods for preparing nuclear extracts are lengthy processes requiring mechanical homogenization, freeze/thaw cycles, extensive centrifugation or dialysis steps that may compromise the integrity of nuclear proteins. The NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit is a reagent-based protocol that enables the stepwise lysis of cells, separation of the cytoplasm from the intact nuclease and then extraction of nuclear proteins away from genomic DNA and mRNA. This gentle process takes less than two hours and requires only a standard bench top centrifuge when using cultured cells. Furthermore, both active nuclear proteins and cytoplasmic proteins can be recovered from the same cell culture or tissue sample.

From two million cells, typical cytoplasmic protein yield is 200 to 500 µg and typical nuclear protein yield is 100 to 200 µg (at a concentration 1 mg/mL). Typical cross-contamination between cytosolic and nuclear fractions is about 10%. The protein concentration of the nuclear extracts can be manipulated easily by varying the volume of nuclear extraction reagent (NER) used in the extraction without any significant loss in extraction efficiency. Specific extraction of nuclear proteins from cells is central to the success of many gene regulation studies. While a variety of methods exist to isolate nuclei and prepare nuclear protein extracts, most of these are lengthy processes requiring mechanical homogenization, freeze/thaw cycles, extensive centrifugation or dialysis steps that may compromise the integrity of many fragile nuclear proteins. The NE-PER Kit overcomes these problems, providing high-quality, concentrated nuclear protein extracts for use in EMSA and other analysis techniques.

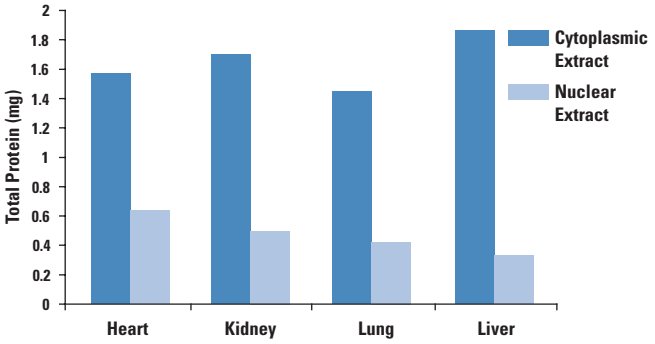


Total protein profile of cytoplasmic and nuclear extracts prepared from a variety of mammalian cell lines using NE-PER Reagents. Protein was quantitated using Thermo Scientific Pierce Micro BCA Protein Assay Reagent (Product # 23235). Values are the average of two separate isolations.

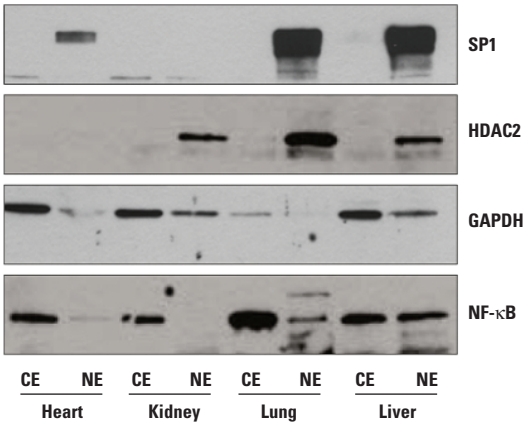


Western blots of specific proteins fractionated using NE-PER into cytosolic extracts (C) and nuclear extracts (N). Two million A549 cells were lysed using Pierce Nuclear and Cytoplasmic Extraction Reagent Kit (NE-PER). Samples were normalized for protein concentration using Pierce BCA Protein Assay. Ten µg of each cytosolic and nuclear extract sample was analyzed by 4-20% SDS-PAGE and Western blotted using specific antibodies diluted 1:1000 (HSP90, Ran, MEK1, MSH2, MCM2, HDAC1, DDX3, Topo IIβ, NF-κB) or 1:10000 (p53, GSK3β, β-catenin, GAPDH). Anti-mouse (H+L) HRP or anti-rabbit (H+L) HRP diluted 1:25,000 was used as the secondary antibody with Thermo Scientific SuperSignal West Dura Chemiluminescent Substrate for detection.

Electrophoretic Mobility Shift Assays (EMSA)



Total protein profile of cytoplasmic and nuclear extracts prepared from different mouse tissues. Swiss Webster mouse tissues (40 mg) were harvested, rinsed with PBS and lysed using the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit. Extracts were quantified using the Pierce 660 nm Protein Assay Reagent (Product # 22660). Values are the average of two separate isolations.



Western blots of specific proteins from fractionated tissues. Cytoplasmic and nuclear extract (10 µg each) from different mouse tissue fractionated using the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit was analyzed by 4-20% SDS-PAGE and Western blotting. Primary antibodies specific for the target proteins were diluted 1:1,000 (SP1, HDAC2 and NF-κB p65, or 1:10,000 (GAPDH). Anti-Rabbit (H+L) HRP (Product # 31460) diluted 1:25,000 was the secondary antibody and SuperSignal® West Dura Chemiluminescent Substrate (Product # 34076) was used for signal detection.

Ordering Information

Product #	Description	Pkg. Size
78833	**NE-PER Nuclear and Cytoplasmic Extraction Reagents** *Sufficient for 50 samples each containing 2 million cells (20 µL packed).*	Kit
78835	**NE-PER Nuclear and Cytoplasmic Extraction Reagents** *Sufficient for 250 samples each containing 2 million cells (20 µL packed)*	Kit

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LightShift Chemiluminescent DNA EMSA Kit

Identifies regulatory sequences and determines protein:DNA binding regions and affinity.

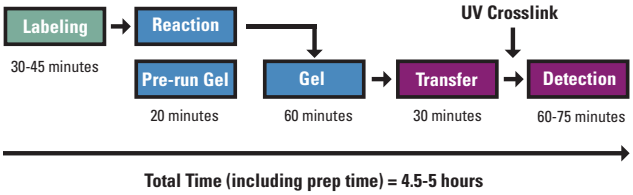
Thermo Scientific LightShift Chemiluminescent DNA EMSA Kit is an extraordinarily robust and sensitive system for performing electrophoretic mobility shift assays (EMSAs) to identify and characterize protein:DNA binding interactions. The kit includes reagents for setting up and customizing protein:DNA binding reactions, a control set of DNA and protein extract to test the kit system, stabilized streptavidin-HRP conjugate to probe for the biotin-labeled DNA target, and an exceptionally sensitive chemiluminescent substrate module for detection.

The principle for our DNA EMSA Detection Kit is similar to that of a Western blot. Biotin end-labeled duplex DNA is incubated with a nuclear extract or purified factor and electrophoresed on a native gel. The DNA is then rapidly (30 minutes) transferred to a positive nylon membrane, UV-crosslinked, probed with streptavidin-HRP conjugate and incubated with the substrate. The protocol from labeling to results can be accomplished in a single day.

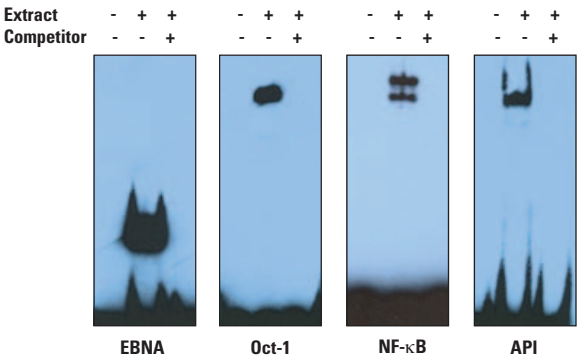
The only additional components needed to perform the assay are purified DNA target that has been end-labeled with biotin, the protein extract you wish to test, nylon membrane and basic electrophoresis equipment. DNA targets may be synthesized with 5´ or 3´ biotin labels or they may be labeled after synthesis using the Biotin 3´ End DNA Labeling Kit (see Product # 89818). Nuclear, cytosolic or whole cell protein extracts may be obtained by a variety of methods, including the Thermo Scientific NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Product # 78833).

Highlights:

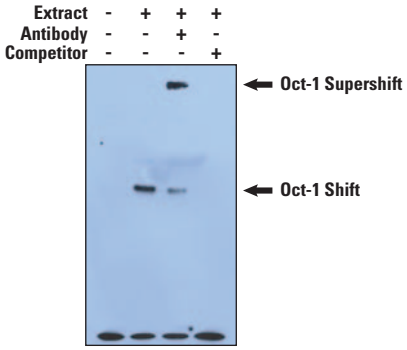
- Includes EBNA control system to help new users develop a working assay and understand the methods used to confirm binding interaction specificity
- Excellent for detecting low-abundance proteins in nuclear extracts
- Sensitivity that surpasses radioactive and digoxigenin methods
- Compatible with previously-established binding conditions for popular DNA:protein interactions



Timeline for the Thermo Scientific LightShift Chemiluminescent DNA EMSA Kit protocol.



Chemiluminescent DNA EMSA of four different DNA:protein complexes. Biotin-labeled target duplexes ranged in size from 21-25 bp. The EBNA reactions were supplemented with 2.5% glycerol and 0.05% NP-40, and the AP1 reactions were supplemented with 10% glycerol. The source of the Oct-1, AP1 and NF-κB transcription factors was a HeLa nuclear extract. EBNA-1 extract is provided as a control in the kit. Unlabeled specific competitor sequences (where used) were present at a 200-fold molar excess over labeled target. X-ray film exposure times for each system ranged from 2 minutes for EBNA, Oct-1 and AP1, and 5 minutes for NF-κB.



The LightShift EMSA Kit has been shown to work with supershifts. LightShift EMSA using a 22 bp Oct-1 specific duplex and a HeLa cell extract. Rabbit anti-Oct-1 antibody (1 µg) was added to the binding reaction last and incubated at room temperature for 20 minutes before loading the native polyacrylamide gel. Film exposure time was 2 minutes.

Ordering Information

Product #	Description	Pkg. Size
20148	**LightShift Chemiluminescent DNA EMSA Kit** *Sufficient components for 100 binding reactions and detection reagents for ~800 cm² of membrane.*	Kit
	Includes: 10X binding buffer	1 ml
	Biotin-EBNA control DNA	50 µl
	Unlabeled EBNA DNA	50 µl
	EBNA extract	125 µl
	Poly(dI•dC)	125 µl
	50% glycerol	500 µl
	1% NP-40	500 µl
	1 M KCl	1 ml
	100 mM $MgCl_2$	500 µl
	200 mM EDTA, pH 8.0	500 µl
	5X Loading buffer	1 ml
	Stabilized streptavidin-horseradish peroxidase conjugate	1.5 ml
	Luminol/Enhancer solution	80 ml
	Stable peroxide solution	80 ml
	Blocking buffer	500 ml
	4X Wash buffer	500 ml
	Substrate equilibration buffer	500 ml

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LightShift Chemiluminescent RNA Electrophoretic Mobility Shift Assay

Fast, non-radioactive detection of RNA:protein interactions

RNA:protein interactions are critical in both the translation of mRNA into protein and in regulatory roles of non-coding RNA. Many studies have focused on the significance of non-coding RNAs, including the 5´ and 3´ untranslated regions (UTR) of mRNA, small interfering RNA (siRNA) and microRNA (miRNA) families. Recently, some of these small RNAs have been implicated as proto-oncogenes and in various diseases and cancer. For both coding and non-coding RNA, RNA:protein interactions are critical for cell function; however, tools for studying such interactions have been limited by the use of radioactivity and high background and high experimental variability.

The Thermo Scientific LightShift Chemiluminescent RNA EMSA Kit provides a non-radioactive solution for studying RNA:protein interactions using an electrophoretic mobility-shift assay (EMSA). An RNA EMSA is an *in vitro* technique that detects protein-RNA interactions through changes in gel electrophoresis migration patterns (Figure 5). A labeled RNA probe is incubated with a protein sample to initiate binding. Once a complex is formed, the sample is separated via non-denaturing polyacrylamide gel electrophoresis. An RNA:protein complex migrates more slowly than a free RNA probe, which shifts the migration pattern. Specificity is determined through binding competition in which excess unlabeled RNA is incubated in the binding reaction, decreasing the signal of the specific interaction.

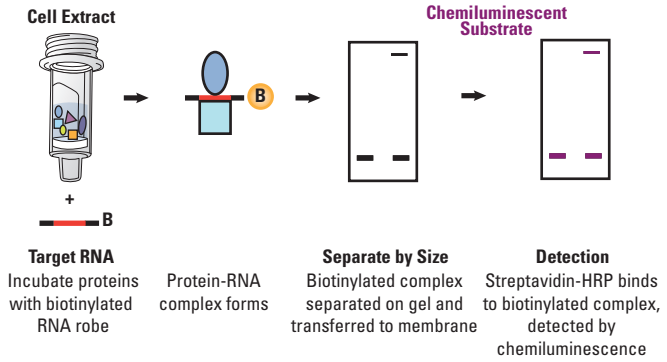

Figure 5. Schematic of the RNA electrophoretic mobility shift assay.

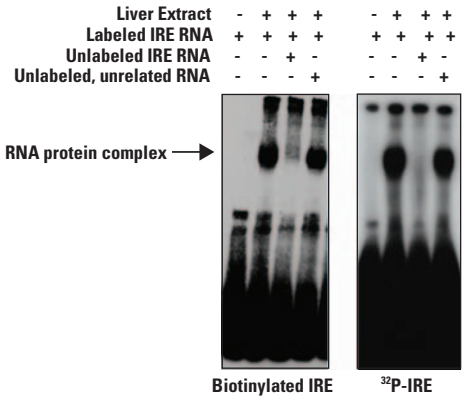

Figure 6. The IRE/IRP RNA EMSA positive control is sensitive and specific. Biotinylated IRE: For the binding buffer reaction, 5 nM (5.1 fmol) of biotinylated IRE RNA was incubated with 4 µg of cytosolic liver extract (containing IRP) in 1X REMSA Binding Buffer, 5% glycerol, and 2 µg of tRNA for 30 minutes at room temperature. Unlabeled RNA (1 µM) was added for the competition reaction, and an unrelated unlabeled RNA (telomerase RNA) was added to demonstrate specificity. Reactions were resolved on a native 6% polyacrylamide gel in 0.5X TBE and transferred to a nylon membrane. Band shifts were detected using the chemiluminescent detection module. **^{32}P-IRE:** A functional gel-shift assay was performed using ^{32}P-end-labeled IRE (5 nM), liver cell extract (5 µg) and the same biotinylated-IRE binding buffer conditions as described above. Densitometry was performed on the scanned gels.

Highlights:

- **Sensitive** – chemiluminescent detection is comparable to radioactive detection
- **Time-saving** – perform the assay from start to finish in less than 1 day
- **Flexible** – compatible with RNA labeled by multiple methods
- **Easy to use** – assay is compatible with cell lysates
- **Non-radioactive** – eliminate radioactive waste concerns

The chemiluminescent RNA EMSA kit contains all the reagents needed for enrichment and detection of the protein-RNA interaction. To perform an RNA EMSA, the biotinylated RNA probe of interest and a protein source, either from a cell lysate or *in vitro* translation, are required. Biotinylated RNA probes may be acquired commercially, generated by run-off transcription with biotinylated nucleotide, or labeled with the Thermo Scientific Pierce RNA Biotin 3´ End-labeling Kit (Product # 20160). End-labeled RNA probes ensure minimal interference with RNA secondary structure and protein interactions.

This kit includes a positive control RNA:protein complex, which is formed and detected in parallel with the experimental sample. The positive control system for the RNA EMSA is the IRE (iron-responsive element)/IRP (iron-responsive protein) RNA:protein interaction. The IRP responds to cellular iron status. Under iron-starved conditions, IRP remains bound to the IRE RNA, suppressing translation of the iron-storage protein, ferritin, and transferrin iron receptor; under iron-rich conditions, IRE binding activity is lost, and ferritin and transferrin are translated. This system is ubiquitous and yields a robust band shift (Figure 6). Incubating the positive control reaction with a 200-fold molar excess of unlabeled IRE RNA reduced the band-shift signal by 70%, indicating specificity (Figure 6); however, incubating the control reaction with a similar fold excess of an unrelated RNA did not significantly reduce the band shift (Figure 6). These results demonstrate the robustness, sensitivity and specificity of the IRE/IRP positive control.

In addition to avoiding radio-labeled nucleotides, a biotinylated RNA probe provides comparable sensitivity to radioactivity with faster detection. The sensitivity of biotinylated RNA probe was compared to a radio-labeled probe by performing RNA EMSAs with different probe concentrations. Both biotinylated and radio-labeled probe band shifts were robust and specific (Figure 7); however, the signal was amplified using the biotinylated IRE-RNA probe after a short exposure (20 minutes) when compared to the band-shift signal and exposure (16 hours with intensifying screen) with the ^{32}P-labeled probe. Both labeling methods were sensitive to the attomole range (Figure 7).

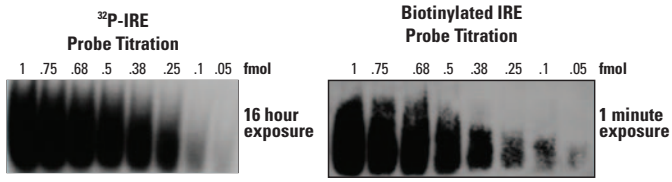

Figure 7. Chemiluminescent detection has comparable sensitivity to radioactive detection. The biotinylated IRE RNA probe was diluted to 50 amol. To generate ^{32}P-IRE, IRE (200 pmol) was labeled with γ-^{32}P ATP using T4 polynucleotide kinase, and then diluted to the same concentration as the biotinylated RNA.

Electrophoretic Mobility Shift Assays (EMSA)

To demonstrate the flexibility of the optimization buffers and assay system, three known protein-RNA interactions were tested: 1) Telomerase RNA (hTR; 451 nucleotides) with telomerase reverse transcriptase (TERT); 2) Let-7 miRNA (100 nucleotides) and the Lin28 protein; and 3) RNA template for RNA polymerase (42 nucleotides) and bacterial RNA polymerase core enzyme. The hTR and Let-7 RNA were labeled using run-off transcription and biotinylated UTP. For the RNA polymerase-binding reaction, the RNA template was end-labeled using the Pierce RNA 3′ End Biotinylation Kit (Product # 20160). TERT and Lin28 proteins were obtained from over-expression lysates (OriGene Technologies). The RNA polymerase was purified from bacteria. The resulting band shifts (Figure 8) demonstrate that the binding buffer, accessory components, and detection module are suitable for various RNA:protein interactions using different RNA labeling methods and protein sources.

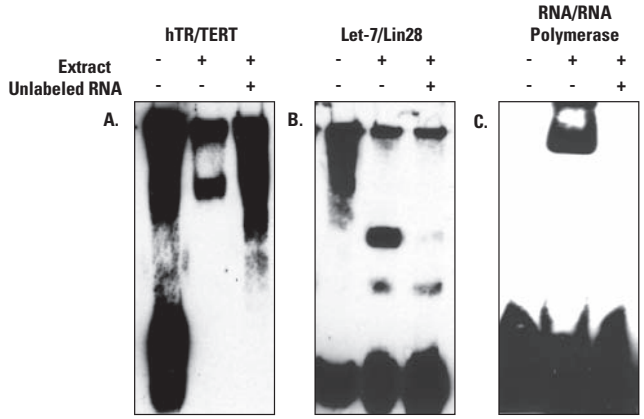

Figure 8. Chemiluminescent REMSA accommodates different RNA and protein sources. Run-off transcription: Plasmid constructs were generated, and run-off transcription was performed using biotin-11-UTP or unlabeled UTP for hTR and Let-7. **3′-end-labeling:** RNA for the RNA polymerase reaction was end-labeled using T4 RNA ligase and a modified biotinylated cytidine. RNA probes were purified and incubated with lysates (TERT, Lin28) or purified RNA polymerase in 1X Binding Buffer. For Let-7/Lin-28, additional DTT and KCl were added, and the glycerol concentration was 2.5% for hTR/TERT. A 50- to 100-fold molar excess of unlabeled RNA was used for the competition reactions. **A.** hTR/TERT, **B.** Let-7/Lin28, **C.** RNA/RNA polymerase.

The LightShift Chemiluminescent RNA EMSA Kit performed comparably to radio-labeled EMSA. The advantages of the system include the flexibility, sensitivity, specificity, and use of non-radiolabeled RNA. The kit works robustly in a variety of systems, including coding and non-coding RNA:protein interactions, various RNA lengths and labeling methods, and purified protein as well as cell lysates.

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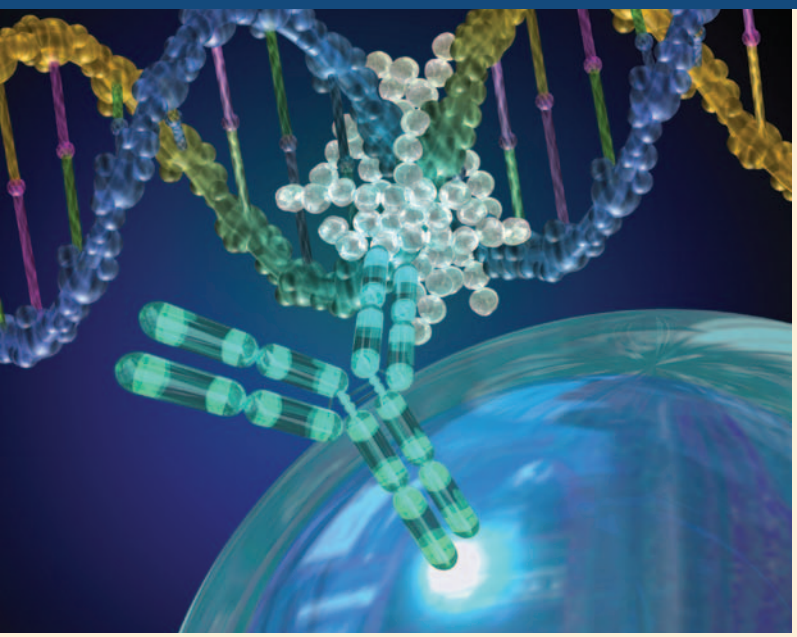
Ordering Information

Product #	Description	Pkg. Size
20158	**LightShift Chemiluminescent RNA EMSA (REMSA)** *Sufficient components for 100 binding reactions and sufficient detection reagents for approximately 1,000 cm² of membrane.* Includes: Biotinylated IRE RNA Control Unlabeled IRE RNA Control Cytosolic Liver Extract tRNA REMSA Binding Buffer (10X) Glycerol, 50% KCl, 2 M $MgCl_2$, 1 M DTT, lyophilized Nuclease-Free Water REMSA Loading Buffer (5X) Stabilized Streptavidin-Horseradish Peroxidase Conjugate Luminol/Enhancer Solution Stable Peroxide Solution Nucleic Acid Detection Blocking Buffer Wash Buffer (4X) Substrate Equilibration Buffer	Kit

Related Products

Product #	Description	Pkg. Size
20159	**tRNA, 10 mg/ml**	100 ml
77016	**Biodyne B Nylon Membranes, 0.45 µm, 8 cm x 12 cm** *Surface modified with positive zeta potential.*	25 sheets
89880	**Chemiluminescent Nucleic Acid Detection Module**	Kit
	Includes: Stabilized Streptavidin-Horseradish Peroxidase Conjugate	1.5 ml
	Luminol/Enhancer Solution	80 ml
	Stable Peroxide Solution	80 ml
	Nucleic Acid Detection Blocking Buffer	500 ml
	Wash Buffer (4X)	500 ml
	Substrate Equilibration Buffer	500 ml

Chromatin Immunoprecipitation (ChIP)



Chromatin immunoprecipitation assays (ChIP assays) identify links between the genome and the proteome by monitoring transcription regulation through histone modification or transcription factor:DNA binding interactions. The strength of ChIP assays is their ability to capture a snapshot of specific protein:DNA interactions occurring ***in vivo***. Additionally, ChIP allows for measurement of relative changes in protein:DNA interaction levels using quantitative polymerase chain reaction (qPCR).

ChIP and Epigenetics

While traditional gene regulation involves DNA sequences adjacent to a gene, Epigenetics is the regulation of gene expression from non-genetic factors. The most commonly studied factors are those which remodel chromatin, thus making stretches of DNA more available for or prohibited to the transcription machinery. The ChIP assay is a common tool to monitor DNA binding of proteins which regulate chromatin structure.

Chromatin structure and remodeling is dependent upon a well studied class of protein, the histones. There are 5 major histone family members, H2A, H2B, H3 and H4 which form an octet that serves as the scaffold for chromatin and H1 which is located in the linker region between DNA wrapped histone octomers. Genomic double stranded DNA wraps around the histone octomer in 160 base pair turns with associated histone monomers binding in the intergenic regions. In addition to forming the higher order chromatin structure necessary for proper chromosome segregation during cell division, histones control access to the DNA by other DNA binding proteins such as transcription factors. Histones are very basic proteins which gives them a net positive charge that enhances the binding of histones to the negatively charged DNA backbone. DNA which is highly associated with histones is in the closed chromatin conformation, or heterochromatin, and undergoes little to no transcriptional activity.

In contrast to heterochromatin, loosely packed DNA, or euchromatin, usually displays high transcriptional activity. The transition between open and closed chromatin is achieved through the modification of histone proteins through acetylation or methylation of lysine and arginine residues. The action of several histone acetyltransferases (HATs) and histone deacetylases (HDACs) add or remove acetyl groups, respectively. Acetylation of N-terminal lysines on the histone surface adds a net negative charge to the protein that repels the DNA backbone and opens the structure of the chromatin. Methylation of histones is regulated by histone methyl transferase and demthylases, which function to add or remove methyl groups, respectively. Histone methylation is commonly associated with transcription repression, however examples exist wherein histone methylation leads to activation of transcription (methylation of histone H3 and H4 is associated with areas of active transcription).

Influences in the cell which affect the post-translational modifications of Histone proteins lead to changes in chromatin structure. ChIP assays using antibodies against modified histones are used to identify these epigenetic events. Using antibodies against modified histones, researchers can use ChIP to correlate the effect of histone modifications on DNA binding.

ChIP and Promoter Profiling

Promoters are DNA sequences upstream of a gene, and are well known regulators of gene transcription. Regulation is due to different classes of proteins binding upstream elements, which regulate the efficiency of transcription of the downstream gene.

The prokaryote genome consists of a single closed circle packaged by histone-like proteins and tethered to the plasma membrane. Prokaryotic, genes are commonly clustered in the same region and share a complex upstream promoter termed an operon. Their genomes contain a very low amount of non-transcribed DNA, due to overlapping ORFs, resulting in greater information to DNA ratio. Prokaryotes employ systems of transcriptional control similar to eukaryotes; however, prokaryotic transcription and translation are coupled events unlike eukaryotes which separate them into discrete cellular compartments. Prokaryotic gene expression is regulated by proteins which exert either a negative and positive effect on transcription. Negative regulation results from a repressor protein binding to a promoter DNA sequence and preventing mRNA transcription. Repressor proteins are regulated by molecular signal called an effector. Positive regulation results from an activator protein binding a DNA promoter which facilitates mRNA transcription. Activator proteins are also regulated by effector molecular signals.

In eukaryotes, the most common transcriptional control is positive regulation through binding of a class of proteins called transcription factors. Because eukaryotic genomic DNA is condensed into chromatin, gene expression only occurs when a stretch of DNA is unraveled (euchromatin) and activated. Activation leads to the binding of RNA Polymerase and subsequent transcription of the downstream gene. Common promoter binding proteins include:

- TATA Binding protein
- DNA Binding Transactivators
- Initiation factors - recruit RNA polymerase
- Elongation factors - phoshphorylate RNA Polymerase

In addition, co-activator protein complexes interact with DNA binding proteins to further regulate transcription. The choreography of proteins binding DNA to regulate gene expression is commonly mapped out using ChIP. ChIP allows researchers to determine which proteins are binding a promoter of interest by using different antibodies.

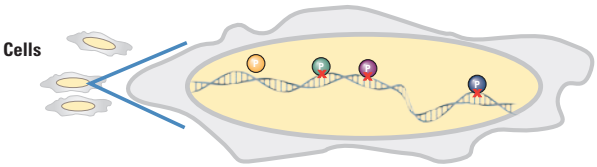
Chromatin Immunoprecipitation (ChIP)

ChIP Assay

A chromatin immunoprecipitation assay can be pieced together using various reagents, or purchased as a fully optimized kit (Product # 26156). Below is an overview of the assay procedure, and a list of reagents which can be used for ChIP.

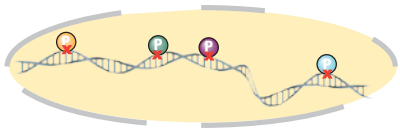
ChIP Assay Procedure

1. Protein:DNA Crosslink with Formaldehyde



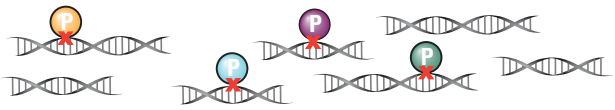
Chromatin immunoprecipitation (ChIP) assays begin with covalent stabilization of protein:DNA complexes. Many protein-DNA interactions are transient, and involve multi-protein complexes to orchestrate biological function. *In vivo* crosslinking covalently stabilizes protein:DNA complexes. *In vivo* crosslinking is traditionally achieved with Formaldehyde (Product # 28906) but can be combined with other crosslinkers such as EGS (Product # 21565) and DSG (Product # 20593). Formaldehyde crosslinking is ideal for two molecules which interact directly. However, formaldehyde is a zero-length crosslinker, limiting it's functionality. For higher order interactions, longer crosslinkers such as EGS (16.1 Angstroms) or DSG (7.7 Angstroms) can trap larger protein complexes with complex quaternary structure. Researchers often use a combination of crosslinkers to trap protein:DNA interacting partners. These crosslinkers permeate directly into intact cells to effectively lock protein:DNA complexes together, allowing even transient interaction complexes to be trapped and stabilized for analysis. See page 25 for a list of references.

2. Cell Lysis



The lysis stage extracts the crosslinked protein:DNA complexes from cells or tissue and brings them into solution. At this stage, cellular components are liberated by dissolving the cell membrane with detergent based solutions. Because protein:DNA interactions occur primarily in the nuclear compartment, removing cytosolic protein can help reduce background and increase sensitivity. The presence of detergents or salts will not affect the protein:DNA complex, as the covalent crosslinking achieved in step one will keep the complex stable throughout the ChIP procedure. Mechanical lysis of cells is not recommended, as it can result in inefficient nuclear lysis. Reagents such as Chromatin Prep Module (Product # 26158), which isolate the nuclear fraction from other cellular components. are used to eliminate background signal and enhance sensitivity.

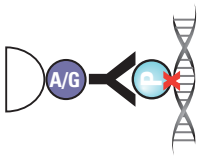
3. Chromatin Digestion



The extraction step yields all nuclear material, which includes unbound nuclear protein, full length chromatin and the crosslinked protein:DNA complexes. In order to analyze protein binding sequences, the extracted genomic DNA must be sheared into smaller, workable pieces. DNA fragmentation is usually achieved either mechanically by sonication or enzymatically by digestion with Micrococcal Nuclease (Product # 88216). Ideal chromatin fragments can range from 200 - >1000 bp, however DNA shearing is one of the most difficult steps to control. Sonication provides truly randomized fragments, but limitations include the requirement of dedicated machinery which may need tuning, difficulty in maintaining temperature during sonication and extended hands-on time and extensive optimization steps. Enzymatic digestion with micrococcal nuclease is highly reproducible and more amendable to processing multiple samples, but can lead to variability due to changes in enzyme activity, and the enzyme has higher affinity for inter-nucleosome regions.

4. Immunoprecipitation, Protein A/G Agarose Caputure, and Wash
5. Elution

Immunoprecipitation, Protein A/G Agarose Capture, and Wash



Elution

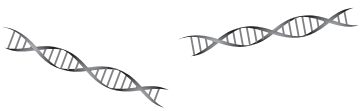
To isolate a specific modified histone, transcription factor or co-factor of interest, ChIP validated antibodies are used to immunoprecipitate and isolate complexes from other nuclear components. This step selectively enriches for the protein:DNA complex of interest, and eliminates all other non-related cellular material. Selection of the appropriate antibody is a critical parameter to successful ChIP assays. For mammalian samples, numerous ChIP-grade antibodies are available which have been validated for this procedure. In non-mammalian species wherein qualified antibodies are unavailable, fusion proteins such as HA, myc, or GST are expressed in the biological sample, and antibodies against these affinity tags are used to immunoprecipitate.

The antibody:protein:DNA complex is affinity purified using an antibody-binding resin such immobilized protein A, protein G or protein A/G. For biotinylated antibodies, immobilized streptavidin or immobilized neutravidin can also be used. For reduced background, it is necessary to block antibody binding beads with a combination of nucleic acid and protein blocking buffers such as salmon sperm DNA and a generic protein source. The volume of beads used in each ChIP sample can also influence background, as the increase in bead volume increases non-specific binding. Optimized antibody binding beads are available, such as ChIP-Grade Protein A/G Plus (Product # 26159). ChIP-Grade Protein A/G Plus is pre-blocked, and has extremely high binding capacity, to mitigate any background threats seen with generic antibody purification resins.

6. Proteinase K Digestion/Crosslink Reversal
7. DNA Purification

Proteinase K Digestion/Crosslink Reversal

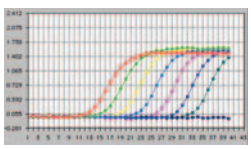
DNA Purification



Enrichment of DNA bound to the protein of interest is the goal for chromatin immunoprecipitation. DNA levels can be determined by agarose gel electrophoresis or more commonly by quantitative polymerase chain reaction (qPCR). Before the specific DNA products of a chromatin IP can be amplified and measured, the crosslinks between protein and DNA must to be reversed. This is typically done through extensive heat incubations, or through digestion of the protein component with Proteinase K (Product # 26160). Proteinase K cleaves at the carboxy-side of aliphatic, aromatic or hydrophobic residues. Because of it's broad specificity, proteinase K is often used for removing proteins form DNA or RNA preps. Additionally, proteinase K digestion eliminates nucleases from the purified DNA, which prevents degradation. To separate DNA from protein fragments, use Phenol Chloroform (Product # 17908) in conjunction with a standard DNA purification method. Alternatively, spin columns designed to purify nucleic acid material from complex biological samples may be used as well.

qPCR Analysis

qPCR Analysis



A hallmark of ChIP is the ability to quantitate the purified DNA products with quantitative PCR. In addition to being an amplification procedure, qPCR accurately measures changes in target protein:DNA levels . There is a direct correlation between the amounts of immunoprecipitated complex and bound DNA. Thermo Scientific AbGene products include a wide variety of qPCR mixes that are compatible with the ChIP method (visit www.thermoscientific.com/abgene for more information).

Using ChIP to Profile the MYC Promoter

A hallmark of the ChIP assay is the ability to quantitate DNA binding by real-time PCR because there is a direct correlation of the immunoprecipitated complex to the bound DNA. We used the Thermo Scientific Pierce Agarose ChIP Kit to profile multiple transcription factors binding to the human MYC promoter in EGF-treated A431 lung carcinoma cells (Figure 9). In this experiment, several transcription factors differentially bound in response to growth factor stimulation. The histone acetyl-transferase CBP does not contain a DNA-binding domain; however, CBP does bind to STAT3, suggesting the increase in CBP binding to the MYC promoter is from recruitment by STAT3, which directly binds DNA. This highlights the sensitivity of the Pierce Agarose ChIP Kit, which was able to profile a difficult-to-detect, second-order protein:protein:DNA interaction *in vivo*.

ChIP Profiling of MYC Promoter Procedure (see Figure 9)

1. 2 x 15 cm dishes of A431 cells were serum starved for 24 hours
2. A431 cells were treated with Epidermal Growth Factor (100 ng/ml) or vehicle control (PBS) for 5 minutes.
3. 16% Formaldehyde was added directly to cells in media (final concentration 1% formaldehyde) to crosslink protein-DNA complexes
4. Crosslinking was quenched with the addition of glycine to a final concentration of 250 µM
5. Cells were rinsed with PBS and lysed using the Chromatin preparation reagents included in the Pierce Agarose ChIP Kit.
6. Chromatin from the two samples (+/- EGF) was collected and sheared using micrococcal nuclease for 15 minutes at 37°C
7. Chromatin from each sample was aliquoted equally into 7 tubes (14 total), resulting in two samples (+/- EGF) for each protein to be analyzed, including the positive control (RNA Pol II) and negative control (Rabbit IgG).
8. One additional aliquot from each treatment equaling 1/10th of the chromatin used for each immunoprecipitation was collected and saved. This input will be used to determine fold-enrichment by comparison to immunoprecipitated samples
9. 1-5 µg of antibody against the following proteins were incubated with an aliquot of chromatin overnight at 4°C.
   a. Phosphorylated STAT3
   b. Acetylated CBP
   c. CBP
   d. Methylated Histone H3
   e. Acetylated Histone H3
   f. RNA polymerase II (10 µl of supplied positive control antibody)
   g. Rabbit IgG (negative control)
10. Antibody complexes were immunoprecipitated using 20 µl of ChIP-Grade Protein A/G Plus Beads for 1 hour at 4°C
11. Protein was digested using 20 mg/ml proteinase K and crosslinking was simultaneously reversed by heating to 65°C for 1.5 hours
12. DNA samples were purified using DNA clean-up columns
13. DNA was added to qPCR reactions with primers for the Myc promoter
14. qPCR levels of enriched samples were compared to the 10% input control, to determine fold enrichment. The signal to input ratio for the different proteins in the presence and absence of EGF were plotted.

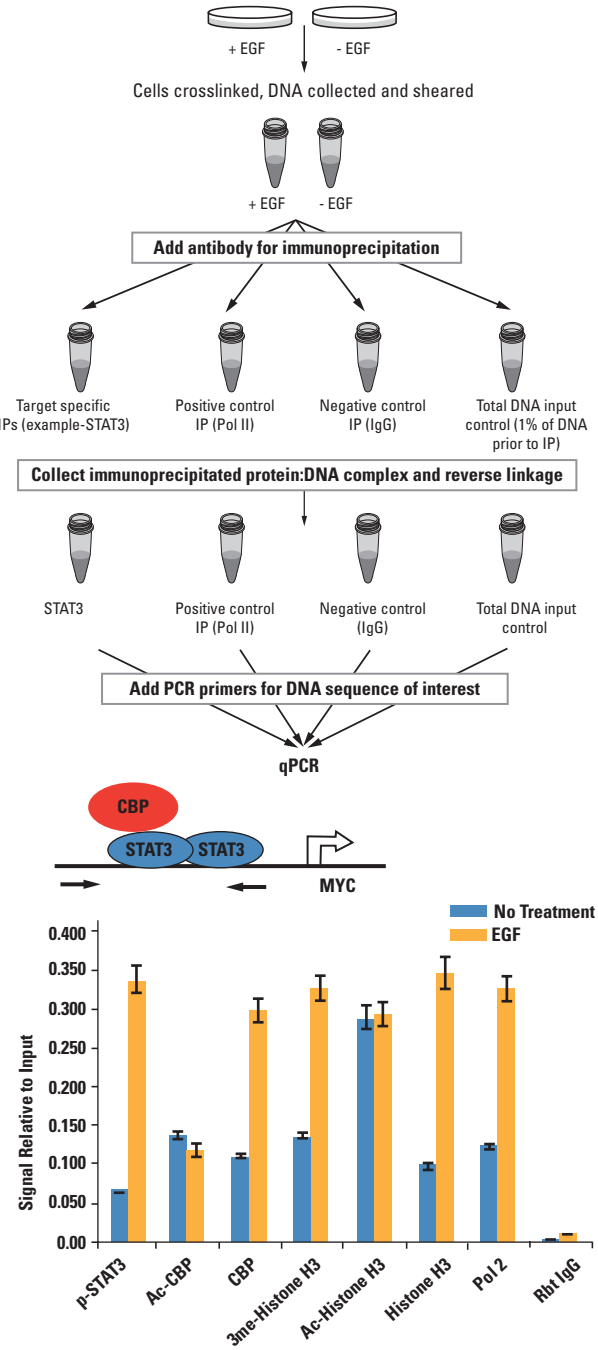


Figure 9. ChIP profiling of MYC promoter using the Thermo Scientific Pierce Agarose ChIP Kit: an effective assay for profiling multiple transcription factors and their binding sites. Crosslinked A431 cells were prepared. Binding of phosphorylated-STAT3 (p-STAT3), acetylated-CBP (Ac-CBP), CBP, trimethyl histone H3 (3me-Histone H3), acetylated-histone H3 (Ac-Histone H3), histone H3, and RNA polymerase II (Pol 2) to the proximal MYC promoter was determined using the Pierce Agarose ChIP Kit. Primary antibody amounts were determined empirically.

References: Use of Crosslinkers Protein:DNA Interactions
(see page 22 for more information)

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ChIP Reagents and Kits

Get highly reproducible ChIP results in 7.5 hours.

Chromatin immunoprecipitation (ChIP) is an effective method for identifying links between the genome and the proteome by monitoring transcription regulation through histone modification (epigenetics) or transcription factor-DNA binding interactions. The strength of the ChIP assay is its ability to capture a snapshot of specific protein-DNA interactions as they occur in living cells, and then quantitate the interactions using standard or quantitative PCR. The Thermo Scientific Pierce Agarose ChIP Kit provides a simple, fast and reproducible method to perform ChIP assays.

Highlights:

- Simple and fast protocol
- Highly efficient isolation and lysis of nuclei
- Easy and reproducible enzymatic digestion
- Low-background and high-binding capacity Protein A/G agarose resin
- Highly specific RNA polymerase II antibody and GAPDH PCR primers included as positive controls
- Fast and reproducible spin-column format
- High-recovery DNA purification

To perform a ChIP assay using the Pierce Agarose ChIP Kit, protein:DNA complexes are immobilized and then extracted. *In vivo* crosslinking is achieved with formaldehyde. Crosslinking, when performed directly in cells, locks in the protein:DNA complexes, trapping these unstable and sometimes transient interactions. To lyse, extract and solubilize the crosslinked complexes, the kit includes the Chromatin Prep Module (also sold separately, Product # 78840). These reagents provide a simple, reliable and convenient means for isolating chromatin-bound DNA without a Dounce homogenizer. With less than 15% contamination from other cellular compartments, the protein of interest is efficiently enriched.

Chromatin Immunoprecipitation (ChIP)

To analyze protein-binding sequences, the genomic DNA must be sheared into smaller, workable pieces, which is usually achieved by sonication or enzymatic digestion. Because sonication often produces variable and hard-to-duplicate results, the Pierce Agarose ChIP Kit includes a specially titrated and tested micrococcal nuclease to digest the DNA. The advantages of enzymatic digestion include reproducibility, reaction control and easy titration of the enzyme for specific cell types (Figure 10).

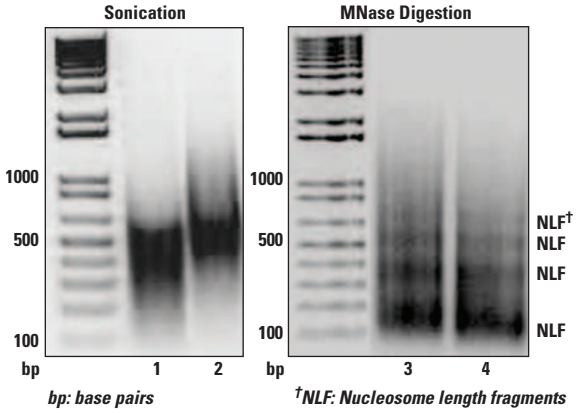

Figure 10. Micrococcal nuclease digestion is more reproducible than sonication for shearing crosslinked chromatin. HeLa cells were cultured in DMEM containing 10% FBS. Crosslinking was achieved using 1% formaldehyde in the media for 10 minutes. Nuclei were isolated using the Thermo Scientific Chromatin Prep Module and divided into four equal samples. Samples 1 and 2 were processed using a Branson Sonifier®, and enzyme digestion was per-formed on samples 3 and 4. Following nuclei lysis, crosslinking was reversed and samples were electrophoresed on a 1.2% agarose gel containing SYBR Green for 1 hour. Gels were imaged with a UV light.

To isolate a specifically modified histone, transcription factor or co-factor, ChIP-validated antibodies are used to immunoprecipitate and isolate specific protein-DNA complexes from other nuclear components. To monitor assay performance, a highly specific ChIP-grade antibody to RNA polymerase II and positive control primers to the GAPDH promoter are provided to determine the relative binding of RNA polymerase II in a control IP. To monitor background, rabbit IgG is included for a negative control IP. The difference in signal between the positive and negative control determine the resolution a ChIP assay can offer to determine changes in protein-DNA complex formation.

Antibody-protein-DNA complexes are recovered using Protein A/G immobilized on agarose resin. The Thermo Scientific Pierce Agarose ChIP Kit uses a specially blocked ChIP-Grade Protein A/G Plus Agarose resin which reduces non-specific binding and provides better enrichment of target protein:DNA complexes (see Figure 11). Additionally, the ChIP-Grade Protein A/G Agarose Resin possesses a higher antibody binding capacity which is a result of improved protein A/G immobilization. This further reduces background because fewer protein A/G beads are needed for each ChIP sample.

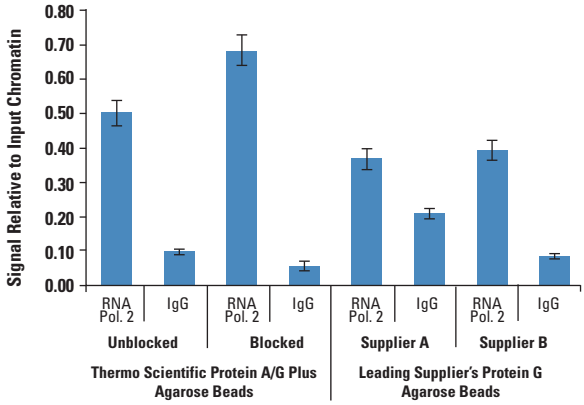

Figure 11. Thermo Scientific ChIP Grade Protein A/G Agarose Beads increase specific signal and decrease background signal in the ChIP assay as compared to ChIP qualified Protein G agarose beads from other leading suppliers. Following formaldehyde crosslinking, HeLa cell lysate was prepared using the Chromatin Prep Module of the Pierce ChIP Assay Kit. Immunoprecipitations were preformed using an anti-RNA Polymerase II (RNA Pol. 2) antibody or Normal Rabbit IgG (IgG). Antibody-antigen complexes were then recovered with blocked or unblocked Pierce Protein A/G Plus Agarose Beads or ChIP Qualified Protein G Agarose beads from two independent suppliers. All other steps in the ChIP assay were performed using the Pierce ChIP Assay Kit. Quantitative PCR was performed using primers which amplify the proximal promoter region of the human GAPDH promoter.

To remove nonspecific protein and DNA binding to the resin, a series of washes is performed followed by an elution of the specific antibody-antigen-DNA complex from the resin. Traditionally, the resin is washed in a microcentrifuge tube, centrifuged and the wash buffer removed by pipetting. The Pierce Agarose ChIP Kit includes spin columns for increasing assay speed and handling convenience, while reducing sample loss.

The DNA:protein link must be reversed before quantitating the immunoprecipitated DNA. The supplied columns and optimized helps to streamline the complete crosslink reversal, protein digestion and DNA purification with minimal time and sample handling. When compared with other suppliers' kits, our kit had greater specific signal and less background (Figure 12).

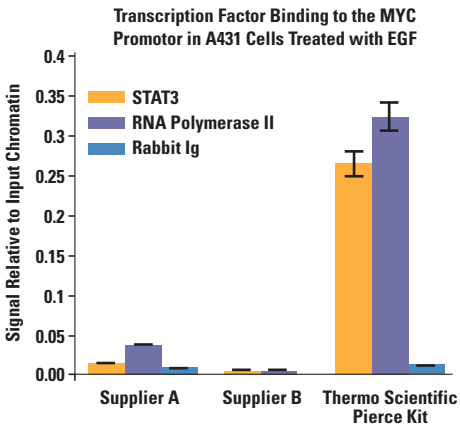

Figure 12. The Thermo Scientific Pierce Agarose ChIP Kit has greater specific signal and less background than other kits. A431 lung carcinoma cells were cultured in DMEM containing 10% FBS for 24 hours. Following a 24 hour serum withdrawal, half of the cultures plated were treated with 100 ng/ml EGF for 10 minutes. Crosslinking was achieved using 1% formaldehyde in the media for 10 minutes. ChIP assays were performed according to the manufacturers' protocols. Quantitative real-time PCR data was obtained with a Bio-Rad iQ5 Thermocycler, ABsolute™ QPCR SYBR® Green Fluorescein master mix, and primers designed to amplify a region of the human MYC promoter proximal to the transcription start site.

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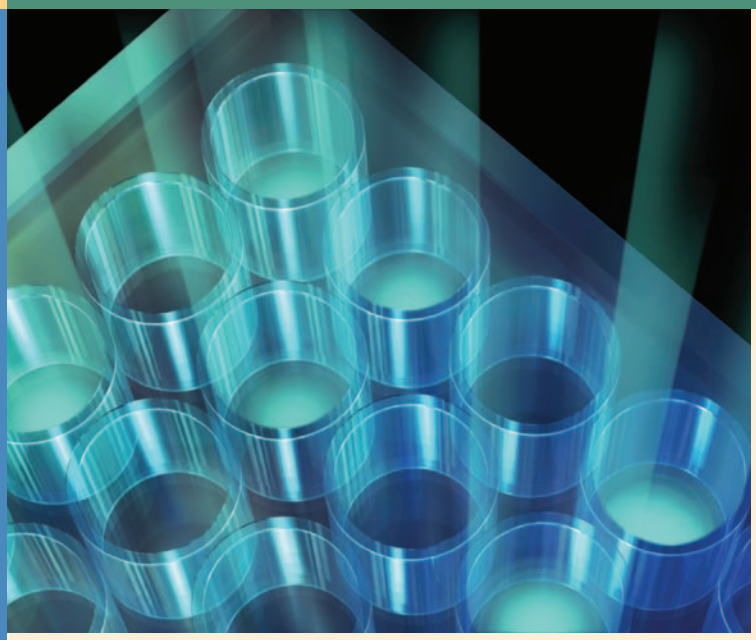
Ordering Information

Product #	Description	Pkg. Size
26156	**Pierce Agarose ChIP Kit**	Kit
	Sufficient reagents for 30 ChIP reactions	
	Includes: ChIP Grade Protein A/G Plus Agarose	0.65 ml
	IP Dilution/Wash Buffer (5X)	11 ml
	IP Wash Buffer 3 (5X)	4.5 ml
	IP Elution Buffer (2X)	4.5 ml
	Column Accessory Pack, 10 spin columns, 20 collection tubes and 10 plugs	3 each
	Microcentrifuge Tubes 75	1.5 ml
	DNA Clean-Up Columns	40
	DNA Column Binding Solution,	30 ml
	DNA Column Wash Solution	6 ml
	pH Indicator	0.8 ml
	DNA Column Elution Solution	5 ml
	Anti-RNA Polymerase II Antibody	25 µl
	Normal Rabbit IgG (1 mg/ml)	10 µl
	ChIP Positive Control Primers (GAPDH promoter)	100 µl
	Pierce Chromatin Prep Module (Product # 26158)	
26158	**Pierce Chromatin Prep Module**	Kit
	Sufficient reagents for 30 chromatin preparations	
	Includes: Membrane Extraction Buffer	15 ml
	Nuclear Extraction Buffer	15 ml
	MNase Digestion Buffer	5 ml
	MNase Stop Solution	0.5 ml
	Halt™ Protease and Phosphatase Inhibitor Cocktail EDTA-free (100X)	4 x 90 µl
	Sodium Chloride (5 M)	3 ml
	Glycine Solution (10X)	15 ml
	PBS (20X)	15 ml
	Micrococcal Nuclease (ChIP Grade) (10 U/µl)	25 ml
	Proteinase K (20 mg/ml), DTT, Lyophilized	0.25 ml 1 vial
26159	**ChIP-Grade Protein A/G Plus Agarose**	0.65 ml
26160	**Proteinase K (20 mg/ml)**	0.25 ml

For a listing of ChIP-grade Pierce Antibodies, please visit www.thermoscientific.com/ab

Transcription Factor Assays



One of the most commonly studied class of protein:DNA interactions are transcription factors. Binding to sequences which flank coding regions, transcription factors regulate gene expression by recruiting or blocking the transcription machinery of the cell to the gene of interest. Different transcription factors share a number of conserved DNA binding domains, however they are able to selectively regulate different genes in response to cellular signaling. The complex regulation of 30,000+ genes from a small number of related transcription factors is believed to be the result of transcription factors "sliding" along the genome to find their target sequence (1) and forming complexes with other co-factors to selectively regulate genes (2).

In vivo transcription factor activity is measured through gene reporter assays. These assays involve the introduction of hybrid gene promoter-reporter gene DNA plasmids into a target cell line. The promoter is specific for a transcription factor of interest, and the reporter gene is a simple enzyme which can produce a detectible signal (such as β-galactosidase or luciferase). When an endogenous transcription factor(s) binds the hybrid promoter, the reporter gene is produced and detected. This is an indirect readout of endogenous gene expression, which can be done in real time with high sensitivity. With multiple transcription factors binding a promoter, gene reporter assays do not resolve which transcription factor is binding. These questions are addressed through alternative methods such as chromatin immunoprecipitation assays (see page 21).

In vitro assessment of transcription factor activity can be done with electrophoretic mobility shift assays (see page 13) or ELISA methods. In this procedure, cell or tissue extracts are collected after treatment, and processed as a typical ELISA. Instead of capture antibodies, double-stranded DNA sequences specific for the target transcription factor coat the 96-well microtiter plate. The DNA probes selectively bind active transcription factors (as inactive transcription factors would not bind DNA), and all non-binding cellular material is removed. Primary antibodies against a specific transcription factor are labeled with common detector moieties (such as horseradish peroxidase) and introduced to each well to detect the presence of a particular transcription factor. *In vitro* ELISA methods identify specific transcription factors, and can be amended to high throughput assays. However, these ELISA methods do not give absolute quantitation because no "active" transcription factor standards for comparison to a cell extract exist. Instead, these ELISA methods deliver quantitative changes in transcription factor binding relative to non-treated controls. Pre-coated transcription factor activation kits are available for commonly studied targets.

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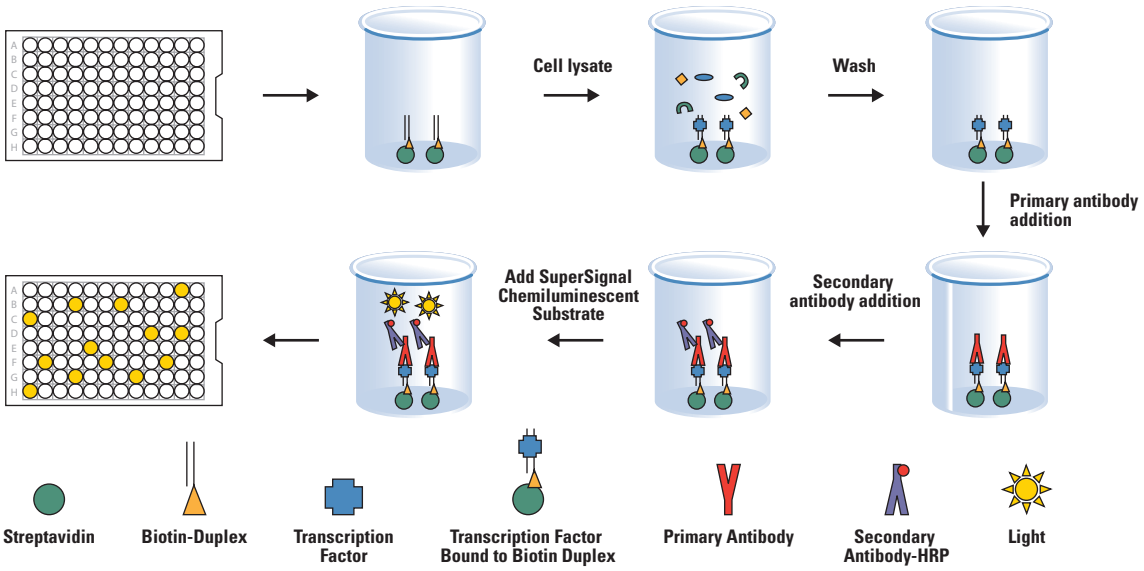
Chemiluminescent Transcription Factor Assay Kits

Measure transcription factors by capturing them using their specific DNA binding sequences.

Thermo Scientific Chemiluminescent Transcription Factor Assay Kits are complete systems containing all the necessary components to measure activated transcription factors in a microplate format. Kits contain streptavidin-coated 96-well plates with the bound biotinylated-consensus sequence for the respective factor. The biotinylated-consensus duplexes bind only the active forms of transcription factors, producing greater signal-to-noise ratios than a traditional ELISA. The captured active transcription factor bound to the consensus sequence is incubated with specific primary antibody (NF-κB p50, NF-κB p65 or c-Fos) then with a secondary HRP-conjugated antibody. After addition of Thermo Scientific SuperSignal Substrate to the wells, a signal is detected using a luminometer or CCD camera.

Transcription factors are proteins that bind to promoter regions of genes to regulate their levels of expression. Defects in transcription factor regulation, structure and/or function have been implicated in numerous human diseases such as cancer and inflammation. The ability to screen for transcription factor activation is, therefore, important to drug discovery as well as to gene regulation studies.

Traditional methods for measuring active transcription factors include gel-shift or electrophoretic mobility shift assays (EMSAs) and colorimetric ELISA-based assays. EMSAs may involve radioactivity and are not amenable to high-throughput applications. Colorimetric ELISA-based assays have increased speed and throughput, but they have limited sensitivity. Thermo Scientific NF-κB p50, NF-κB p65 and c-Fos Transcription Factor Kits are chemiluminescent ELISA-based assays that provide unsurpassed sensitivity and convenience over the existing methodologies.



Summary of Thermo Scientific Pierce Transcription Factor Assay.

Highlights:

- **Sensitivity** – SuperSignal Substrate yields results 20- to 140-times more sensitive than the colorimetric methods with a broader dynamic range
- **Fast** – format allows researcher to perform multiple tests in three to four hours
- **Reliable** – each kit contains a positive control lysate, and wild type and mutant competitor duplexes
- **Compatible with high-throughput systems** – 96-well format increases sample throughput over traditional EMSAs
- **Convenient** – kits contain all the reagents for two 96-well assay plates
- **Versatile** – signal is detectable using a luminometer or CCD Camera

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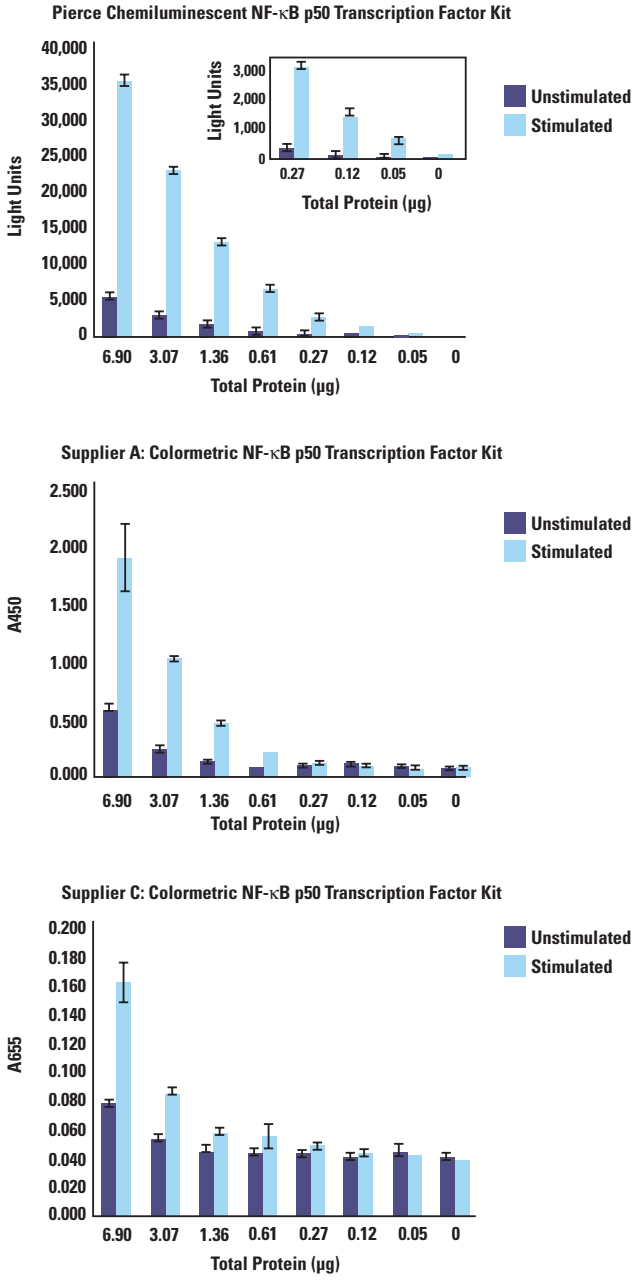
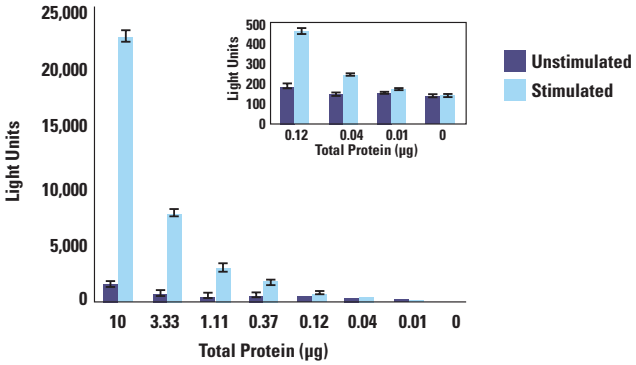
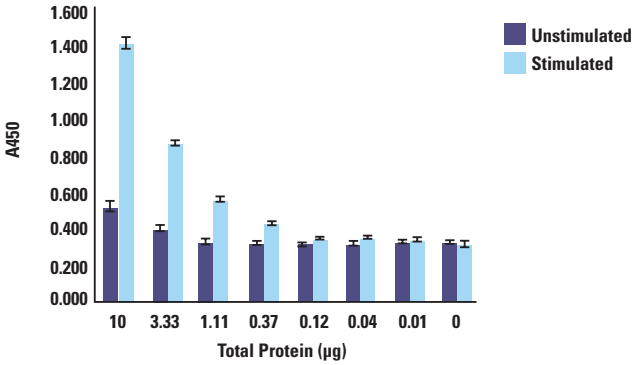


Sensitivity comparison of the Thermo Scientific Pierce NF-κB p50 Kit and other supplier's kits using nuclear extract. Various amounts of TNF-α induced and uninduced HeLa cell nuclear fractions were incubated and detected using the chemiluminescent Pierce NF-κB p50 Kit and colorimetric kits from Suppliers A and C. Chemiluminescent results were detected using a luminometer. Colorimetirc results were detected using a plate reader set to the respective wavelengths based on the manufacturer's product instructions. Error bars represent 1 standard deviation.

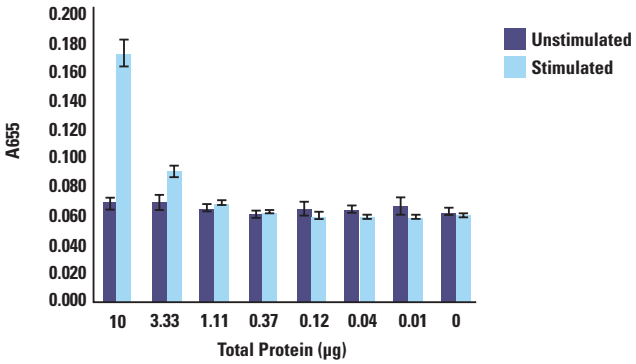
EZ-Detect™ Chemiluminescent NF-κB p65 Transcription Factor Kit



Supplier A: NF-κB p65 Colormetric Transcription Factor Kit



Supplier C: NF-κB p65 Colormetric Transcription Factor Kit



Sensitivity comparison of the Pierce NF-κB p65 Kit and other supplier's kits using whole cell extract. Various amounts of TNF-α induced and uninduced HeLa whole cell extracts were incubated and detected using the chemiluminescent Pierce NF-κB p65 Kit and colorimetric kits from Suppliers A and C. Chemiluminescent results were detected using a luminometer. Colorimetric results were detected using a plate reader set to the respective wavelengths based on the manufacturer's product instructions. Error bars represent 1 standard deviation.

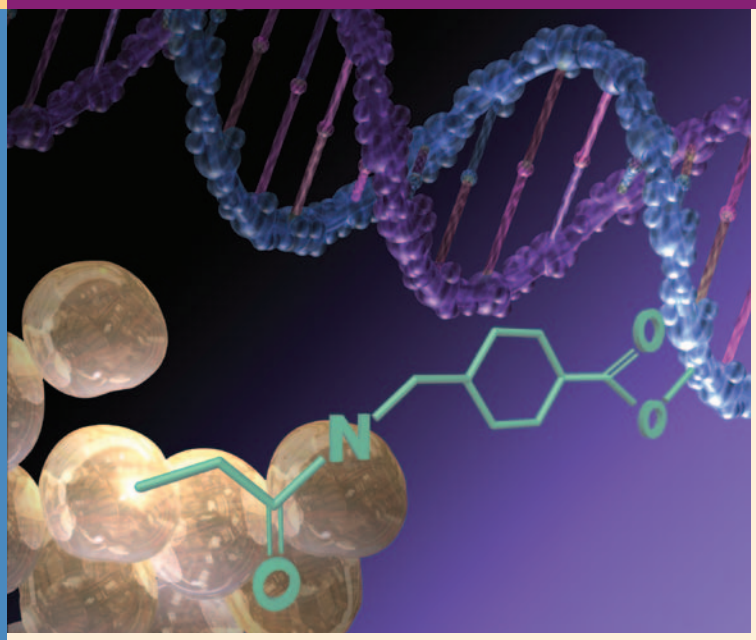
Ordering Information

Product #	Description	Pkg. Size
89858	NF-κB p50 Transcription Factor Kit	2 plates/kit
89859	NF-κB p65 Transcription Factor Kit	2 plates/kit

Each of the above-listed kits contains all reagents needed for two 96-well assay plates.

Includes:	
96-well assay plates	2 plates
Binding buffer (5X)	2 x 1.3 ml
Poly dI•dC (20X)	700 µl
Primary antibody	24 µl
Antibody dilution buffer	60 ml
HRP-conjugated Secondary Antibody	varies
Luminol/Enhancer solution	12 ml
Stable peroxide solution	12 ml
Plate sealers	2
Wash buffer (10X)	48 ml
Wild type and mutant duplex, 10 pmole/µl	72 µl
Activated HeLa cell nuclear extract (Positive Control)	20 µl

Thermo Scientific Protein:Nucleic Acid Conjugates



Crosslinking Protein:Nucleic Acid Interactions

Heterobifunctional reagents are now available that can be applied to the study of site-specific protein:nucleic acid interactions. These reagents are designed to be deployed in a stepwise manner, enabling the capture of a protein:nucleic acid complex. Such conjugations between a protein bait and a nucleic acid prey involve use of crosslinking agents, a subject treated in greater detail as a previous topic in this handbook. Protein:nucleic acid interactions are most often stabilized by linkage with heterobifunctional crosslinkers that have a photo-reactive aryl azide as one of the two reactive groups.

Generally, a purified binding protein is modified by reaction with one of the two reactive groups of the crosslinker. Most reagents target amine functions on the proteins, but other functional groups can be targeted as well. The initial reaction is carried out in the dark since the remaining reactive group of the crosslinker is photo-reactive. The photo-reactive group of the heterobifunctional reagent will ultimately crosslink the site at which the target protein binds when the resulting complex is exposed to light. Typically, the photo-reactive group is an aryl azide-based moiety that can insert nonspecifically upon photolysis.

The modified putative binding protein is incubated with the nucleic acid sample. The complex is captured when exposed to the proper light conditions for the reagent. Band shift analysis can be used to indicate capture of the complex. Alternatively, nucleases can be used to remove those portions of the nucleic acid not protected by the protein binding, thereby isolating the sequence-specific site of interaction. Photo-reactive, heterobifunctional reagents with a cleavable disulfide linkage allow reversal of the protein:nucleic acid crosslink and recovery of the components of the interacting pair for further analysis.

Preparation of Protein:Nucleic Acid Conjugates

The ability to conjugate proteins to nucleic acids, including RNA and DNA, is important in a number of life-science applications. Perhaps the most common conjugate of these molecules made using crosslinking compounds is the labeling of oligonucleotide probes with enzymes. Conjugating enzymes like horseradish peroxidase (HRP) or alkaline phosphatase (AP) to oligos that can hybridize to specific target sequences is important for detecting and quantifying target DNA or RNA. In this application, the enzyme activity is an indicator of the amount of target present similar to immunoassay detection using ELISA techniques. In this case, the oligo probe takes the place of the antibody, but the enzyme assay is detected by substrate turnover in the same manner.

Conjugation to 5´-Phosphate Groups

Using chemical reagents to effect the conjugation of nucleic acids to enzymes can be done using different strategies. A convenient functional group that can be chemically modified to allow the coupling of protein molecules on oligos is the 5´-phosphate group. Using the 5´ end of the oligo as the conjugation point has an advantage of keeping the rest of the nucleic acid sequence unmodified and free so it can easily hybridize to a complementary target. For oligos that have been synthesized, a 5´-phosphate group may be put on the end of the molecule to facilitate this type of conjugation. The alkyl phosphate is reactive with the water-soluble Thermo Scientific Pierce Carbodiimide EDC (Product # 22980, 22981), which forms a phosphate ester similar to the reaction of EDC with a carboxylate group. Subsequent coupling to an amine-containing molecule (i.e., nearly any protein or unmodified peptide) can be done to form a stable phosphoramidate linkage (Figure 13).

If a diamine molecule is used to modify the DNA 5´-phosphate, then the resultant amine-modified oligo can be coupled to enzyme molecules using a heterobifunctional reagent. Using a diamine compound that contains a disulfide (e.g., cystamine) and then reducing the disulfide group results in a sulfhydryl that may be conjugated with proteins rendered sulfhydryl-reactive using the heterobifunctional reagent Sulfo-SMCC (Product # 22322). We offer HRP and AP enzymes that have been made sulfhydryl-reactive (i.e., maleimide-activated) by this mechanism (Product # 31485 and # 31486, respectively).

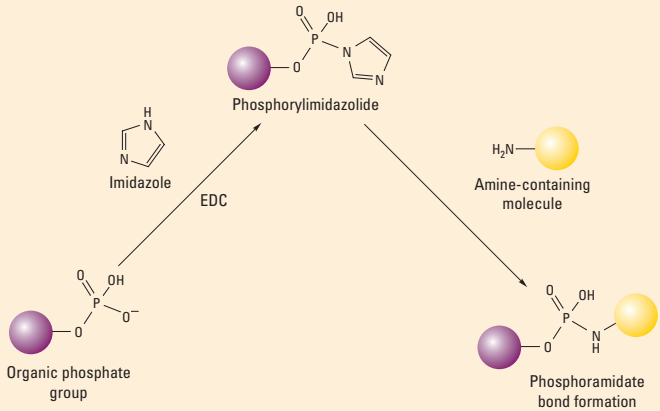


Figure 13. Reaction scheme showing typical route to conjugating an amine-containing biomolecule to a 5´ terminal phosphate group.

Conjugation to the 3´ End of RNA

Alternatively, the 3´ end of RNA molecules may be chemically modified to allow coupling with amine-containing molecules or proteins. The diol on the 3´-ribose residue may be oxidized to result in two aldehyde groups using Thermo Scientific Sodium *meta*-Periodate (Product # 20504). The aldehydes then can be conjugated to the amine groups on a protein using reductive amination with Thermo Scientific Sodium Cyanoborohydride (Product # 44892). The aldehyde and the amine first form a Schiff base that is reduced to a secondary amine linkage with the cyanoborohydride reductant.

Biotinylation of Nucleic Acids

Nucleic acid molecules also can be biotinylated by a number of chemical methods. Using the strategies previously described to modify the 5´ or 3´ ends of oligos with a diamine (e.g., Product # 23031) will provide a functional group that can be reacted with any amine-reactive biotinylation compound, such as Thermo Scientific Sulfo-NHS-LC-Biotin (Product # 21335). This modification method would provide a biotin group at the end of an oligo probe, thus allowing streptavidin reagents to be used to detect a hybridization event with a target.

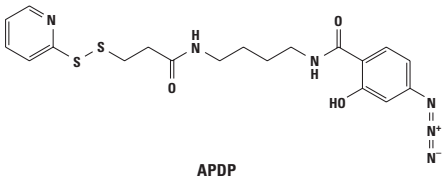
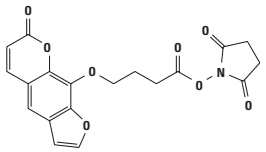
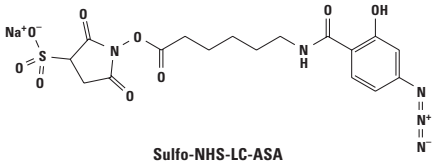
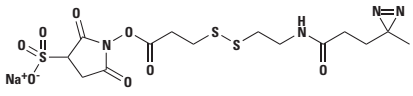
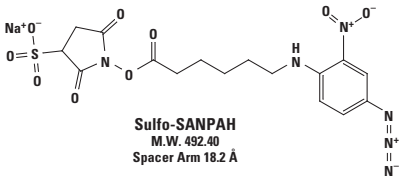
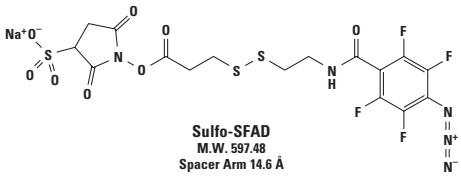
Biotinylation of oligonucleotides can also be done using photo-reactive reagents. There are two main options commonly used to add one or more biotin residues to nucleic acid probes. Thermo Scientific Photoactivatable Biotin (Product # 29987) contains a phenyl azide group at the end of a spacer arm with the biotin group at the other end. Photolyzing a solution of the biotin compound together with an oligo in solution results in biotin being nonselectively inserted into the nucleic acid structure. Alternatively, Thermo Scientific Psoralen-PEG-Biotin (Product # 29986) can be used to label double-stranded DNA or RNA. The psoralen ring structure effectively intercalates into the double-stranded portions, and exposure to UV light causes a cyclo-addition product to be formed with the 5,6-double bond in thymine residues. The poly(ethylene oxide) spacer in Psoralen-PEG-Biotin contributes excellent water solubility, thus assuring that the resultant derivative will have accessibility to streptavidin-containing detection reagents.

Additionally, enzymatic biotinylation of DNA (Product # 89818) or RNA (Product # 20160) provides 3' end labeling. This method reduces interference of complex formation, which can occur with internal biotin labeling (see pages 10-11).

Crosslinking Agents

This section covers the Thermo Scientific Pierce Crosslinking Reagents that can be applied to the preparation of nucleic acid-based conjugates, including biotinylation and the study of protein:nucleic acid interactions.[1,2,3] Heterobifunctional reagents can be applied to the study of site-specific protein:nucleic acid interactions. These reagents are designed to be deployed in a stepwise manner enabling the capture of a protein:nucleic acid complex. Reagents that have been applied or with potential application to the study of protein:nucleic acid interactions are listed on the next page.

Ordering Information

Product #	Product Name	Structure	Key Features	Ref.	Pkg. Size
27720	**APDP** *N*-[4-(*p*-Azido-salicylamido) butyl]-3′-(2′-pyridyldithio) propionamide	 **APDP** M.W. 446.55 Spacer Arm 21.0 Å	• –SH group reactive • Iodinatable • Cleavable • Reaction with nucleic acid binding protein monitored at 343 nm	4	50 mg
23013	**SPB** Succinimidyl-(4-[psoralen-8-yloxy])-butyrate	 **SPB** M.W. 385.32 Spacer Arm 8.9 Å	• Amine-reactive • DNA intercalacting agent • Psoralen couples via 5 or 6 member end rings	7,8,9	50 mg
27735	**Sulfo-NHS-LC-ASA*** Sulfosuccinimidyl (4-azido-salicylamido)hexanoate **See also: NHS-ASA (Product # 27714)*	 **Sulfo-NHS-LC-ASA** M.W. 491.41 Spacer Arm 18.0 Å	• Can incorporate ^{125}I label before acylation step • Photolysis initiated by long-wave UV • Water-soluble • Non-cleavable • Amine-reactive	10	50 mg
26175	**Sulfo-SDAD** (sulfosuccinimidyl 2- [(4,4′-azipentanamido) ethyl]-1,3′-dithiopropri-onate)	 **Sulfo-SDAD** Sulfo-NHS-SS-Diazirine Spacer Arm 13.5 Å			50 mg
22589	**Sulfo-SANPAH*** Sulfosuccinimidyl 6-(4-azido-2′-nitrophenyla-mino) hexanoate **See also: SANPAH (Product # 22600)*	 **Sulfo-SANPAH** M.W. 492.40 Spacer Arm 18.2 Å	• Optimal photolysis occurs at 320-350 nm limiting damage to biomolecules by irradiation • Water-soluble • Non-cleavable • Amine-reactive	12	50 mg
27719	**Sulfo-SFAD** Sulfosuccinimidyl(perfluoro azidobenzamido)ethyl-1,3′-dithiopropionate	 **Sulfo-SFAD** M.W. 597.48 Spacer Arm 14.6 Å	• Improved photoconjugation, efficiency • Photolyzes at 320 nm • Water-soluble • Cleavable • Amine-reactive	5,6	50 mg

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Alkaline Phosphatase

Ready-to-conjugate preparation.

Highlight:

- Specific activity 2,000 units/mg

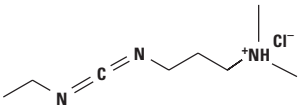
Ordering Information

Product #	Description	Pkg. Size
❄ 31391	Alkaline Phosphatase	20 mg
❄ 31392	Alkaline Phosphatase	100 mg

❄ *Additional dry ice and/or freight charge.*

EDC

Activates phosphate groups on oligos.



EDC
M.W. 191.70

Highlight:

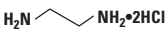
- Water-soluble carbodiimide that activates phosphate groups on oligos and carboxylate groups on proteins similarly. Coupling occurs via amine groups on enzymes such as HRP or AP, forming a stable phosphoramidate linkage.

Ordering Information

Product #	Description	Pkg. Size
22980	EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride)	5 g
22981	EDC	25 g

Ethylenediamine Dihydrochloride

Used in 3´ or 5´ end labeling strategies for oligos.



Ethylenediamine
M.W. 133.02

Ordering Information

Product #	Description	Pkg. Size
23031	Ethylenediamine Dihydrochloride	10 g

Horseradish Peroxidase

High-specific activity preparation ideal for protein:nucleic acid conjugate preparations.

Highlights:

- Small high-turnover rate enzyme of 40K MW
- Conjugates compatible with a number of substrates

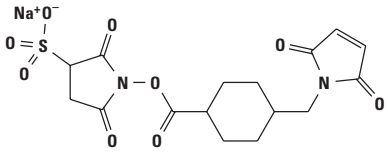
Ordering Information

Product #	Description	Pkg. Size
❄ 31490	Horseradish Peroxidase	10 mg
❄ 31491	Horseradish Peroxidase	100 mg

❄ *Additional dry ice and/or freight charge.*

Sulfo-SMCC

Yields stable protein:nucleic acid conjugates.



Sulfo-SMCC
M.W. 436.37
Spacer Arm 8.3 Å

Highlights:

- Couples readily to an amine-derivatized oligo by EDC coupling of a diamine to the 5´ phosphate group
- Nucleic acid can be sequentially coupled to an available –SH group on a protein/enzyme

Ordering Information

Product #	Description	Pkg. Size
22322	Sulfo-SMCC (Sulfosuccinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate)	50 mg

Sodium *meta*-Periodate

An oxidation agent of choice for creating active aldehydes from 3´ ribose diols in RNA.

Highlight:

- Aldehydes can be conjugated to amine groups on a protein or enzyme using reductive amination with sodium cyanoborohydride

Ordering Information

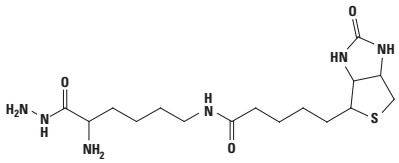
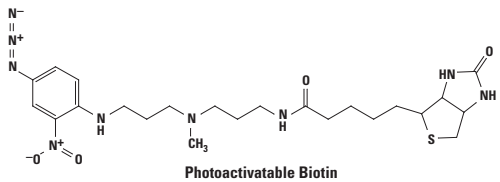
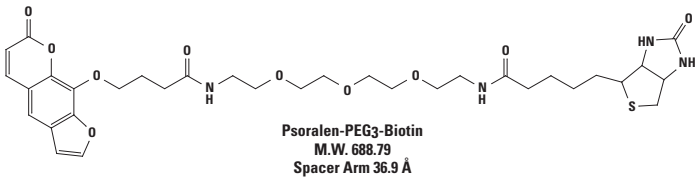
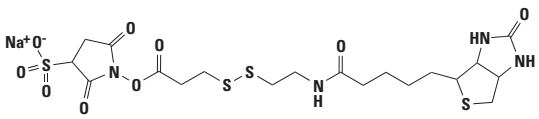
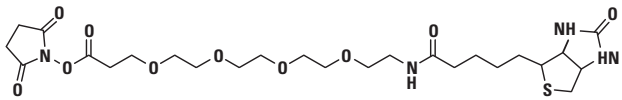
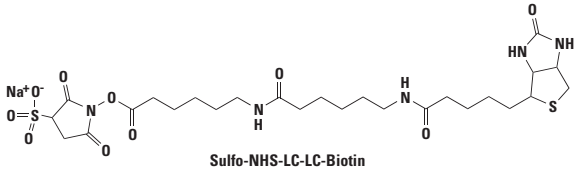
Product #	Description	Pkg. Size
✖ 20504	Sodium *meta*-Periodate	25 g
✖ 44892	AminoLink® Reductant (Sodium cyanoborohydride)	2 x 1 g

✖ *Additional hazardous charge.*

Nucleic Acid-Biotin Conjugates

See the biotinylation section at www.thermoscientific.com/pierce for our complete selection of Thermo Scientific EZ-Link Reagents.

Ordering Information

Product #	Product Name	Structure	Key Features	Ref.	Pkg. Size
28020	Biocytin Hydrazide	 Biocytin-Hydrazide M.W. 386.51 Spacer Arm 19.7 Å	• Carbohydrate-reactive • Used to label DNA and RNA through cytosine • Extended spacer arm • Forms hydrazone bond • Water-soluble	1	25 mg
29987	Photoactivatable Biotin	 Photoactivatable Biotin M.W. 533.65 Spacer Arm 30.0 Å	• Water-insoluble • Membrane-permeable • Modifies DNA/RNA • Covalent nonspecific coupling occurs in presence of UV light (350-370 mn)	2	0.5 mg
29986	Psoralen-PEG$_3$-Biotin	 Psoralen-PEG3-Biotin M.W. 688.79 Spacer Arm 36.9 Å	• One step labeling • Photoactivate at ~350 nm • Labels DNA/RNA probes/ PCR products/oligos or cDNA inserts • Water-soluble • Promotes conjugate solubility • Does not interfere with hybridization	3-5	5 mg
21331	NHS-SS-Biotin	 Sulfo-NHS-SS-Biotin M.W. 606.69 Spacer Arm 24.3 Å	• Water-soluble • Amine-reactive, forms stable amide bond • Cleavable spacer arm	6	100 mg
21362	NHS-PEG$_4$-Biotin	 NHS-PEG4-Biotin M.W. 588.67 Spacer Arm 29 Å	• Water-soluble spacer arm increases solubility of conjugate • Amine-reactive, forms stable amide bond	--	50 mg
21335	Sulfo-NHS-LC-Biotin	 Sulfo-NHS-LC-LC-Biotin M.W. 669.75 Spacer Arm 30.5 Å	• Water-soluble • Amine-reactive, forms stable amide bond	7	100 mg

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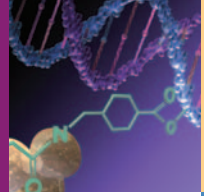

Biotin Random Prime DNA Labeling Kit

A safe alternative to radiolabeling of oligonucleotide probes.

Thermo Scientific North2South Random Prime Labeling Kit is based on the procedure of Feinberg and Vogelstein(Ref.4,5). Random heptanucleotides containing all possible sequences are annealed to a denatured DNA template. These act as primers for complementary strand synthesis by DNA Polymerase (Klenow fragment, 3'-5' exo-). Biotinylated dNTPs in the reaction mix are incorporated into the newly synthesized DNA. This protocol yields biotin-labeled DNA of high activity for use as probes in hybridization experiments such as Southern and Northern hybridizations. The resulting hybrids can be detected with streptavidin-horseradish peroxidase (HRP) and a chemiluminescent substrate kit such as the Thermo Scientific Pierce Chemiluminescent Hybridization and Detection Kit.

Highlights:

- Works with as little as 100 ng starting DNA template
- Exonuclease-free Klenow fragment in the DNA labeling kit produces higher yields
- Each reaction yields probe sufficient for approximately three (10 x 10 cm) blots

References

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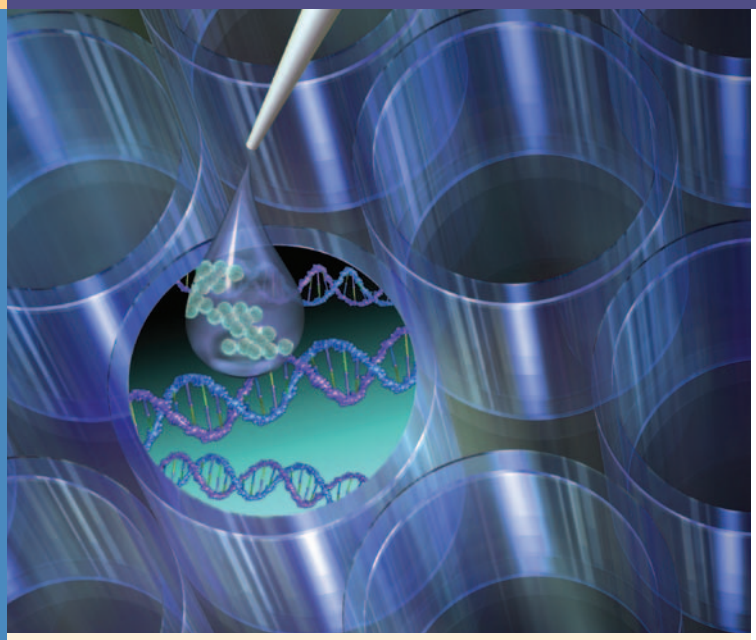
5. Feinberg, A.P. and Vogelstein, B. (1983). *Anal. Biochem.* **132**, 6.

Ordering Information

Product #	Description	Pkg. Size
17075	**North2South Biotin Random Prime DNA Labeling Kit**	Kit
	*Sufficient reagents for 10 reactions.***	
	Kit contains: 5X Heptanucleotide Mix	100 µl
	5X Deoxynucleotide Mix	80 µl
	10X Reaction Buffer	50 µl
	Klenow DNA Polymerase	10 µl
	Non-biotinylated control DNA	5 µl
	5M Ammonium Acetate	1 ml
	0.5M EDTA	1 ml
	Nuclease-free Water	1 ml
	Biotin-11-dUTP	20 µl

*** Each reaction yields probe sufficient for approximately three 10 x 10 cm blots.*

Affinity Methods for Protein:Nucleic Acid Interactions



Affinity-Capture Methods for Protein:Nucleic Acid Interactions

Access to the latest nucleic acid-sequencing and labeling technologies has been a great asset to *in vitro* affinity methods of verifying and characterizing the interaction of protein with specific nucleic acid sequence motifs. Short nucleic acid oligos (DNA or RNA) encoding the sequence under study are most popularly labeled with amine or biotin tags linked to the 5′ end via a crosslinker. These biotin- or amine-labeled oligos are then amenable to immobilization and detection strategies that allow *in vitro* protein:nucleic acid interaction studies.

Plate Capture Methods

In the literature, there are several ways to immobilize DNA or RNA (bait) and analyze the interaction of specific proteins (prey) with the bait. One popular method uses 96- or 384-well microplates coated with streptavidin to bind biotinylated DNA/RNA baits. A cellular extract is prepared in binding buffer and added for a sufficient amount of time to allow the putative binding protein to come in contact and "dock" onto the immobilized oligonucleotide. The extract is then removed and each well is washed several times to remove nonspecifically bound proteins. Finally, the protein is detected using a specific antibody labeled for detection. This method can be extremely sensitive because the antibody is usually labeled with an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), that amplifies the signal over time according to the label's enzyme activity. Coupling enzymatic amplification of signal with a chemiluminescent substrate suited to ELISA-based applications (e.g., Product # 37070) can lead to detection of less than 0.2 pg of the protein of interest per well. This same ELISA-based method may also be used for amine-labeled oligos using microplates coated with an amine-reactive surface chemistry (e.g., Thermo Scientific Pierce Maleic Anhydride Plates, Product # 15110).

Pull-down (Gel Support) Methods

Another popular affinity-based format for studying protein:nucleic acid interactions *in vitro* is the pull-down method. In this case, as in the ELISA method, the amine- or biotin-labeled nucleic acid is immobilized on either an amine-reactive or immobilized streptavidin gel surface. The gel may be prepared in a spin cup, column or batch format, depending on individual requirements. After the nucleic acid bait has been immobilized, a cellular extract containing the putative prey protein is prepared in binding buffer and incubated for a sufficient time with the immobilized oligonucleotide. Once the gel has been washed thoroughly, the purified protein prey may be eluted from the nucleic acid bait by a stepwise salt gradient or other buffer condition sufficient to disrupt the interaction. After the prey has been eluted, it is amenable to virtually any characterization technique. SDS-PAGE may be performed with the eluted sample, allowing sizing relative to molecular weight standards. It also may be transferred to membrane for more thorough identification by Western blotting. Depending on the method of detection and abundance of the putative DNA or RNA binding protein in the cellular extract, the pull-down technique may require a greater amount of starting material.

Flexible Methods

There are many variations to the ELISA and pull-down methods that do not significantly alter the basic premise of each. For example, the labeled DNA or RNA oligo may be first incubated with the cellular extract and then the entire protein:nucleic acid complex immobilized on the plate surface or gel. In contrast to the sequential binding and washing of the oligo, and the addition of the cellular extract, adding the oligo directly to the cellular extract before binding the nucleic acid:protein complex on the gel surface may solve logistical problems, especially when steric hindrance is suspected.

Current options in gel format selection may also be chosen to fit the requirements of each experimental system, though the assay basics remain similar. For instance, the amine-reactive or streptavidin-coated gel may be placed in a column for standard column chromatography. Additionally, the gel may be processed in a spin cup for use with a microcentrifuge, vacuum manifold or syringe. Alternatively, derivatized magnetic beads may be used to achieve magnetic separation. The list of options is quite extensive and is limited only by the imagination of the researcher and/or the logistics of a particular experiment.

Practical Considerations

Several steps may be taken to reduce the chances of anomalous data generation when working with proteins and nucleic acids in the context of cellular extracts.

1. Remember to always include protease and nuclease inhibitors to decrease the chances of protein and oligo degradation.
2. Take appropriate measures to reduce nonspecific binding of proteins to either the oligo or gel surface. For example, poly(dI•dC) is often included in the cellular extract as a weak competitor to the oligo, and can significantly decrease nonspecific binding events.
3. Make sure to include all cofactors and conditions required for the protein to bind the DNA or RNA. Also, some proteins may require the nucleic acid to be double- or single-stranded before binding can occur.
4. Consider the length of the carbon chain between the biotin or amine label and the oligo. It can make a significant difference, reducing the steric hindrance of the bound oligo.
5. Proper use of controls will be essential to successful execution of any experiment.

Selected Thermo Scientific products for use in the study of protein:nucleic acid interactions.

Product Name	Product #	Product Description
Pierce Pull-Down Biotinylated-Protein:Protein Interaction Kit	**21115**	Each kit allows 25 pull-down assays in a spin column format, standard buffer system included. Successfully used for nucleic acid:protein interactions.
Streptavidin Agarose Resin	**20347** (2 ml gel) **20349** (5 ml gel) **20353** (10 ml gel)	Binds 15-28 µg biotin per ml of gel (1-3 mg biotinylated BSA per ml of resin).
High Capacity Streptavidin Agarose	**20357** (2 ml gel) **20359** (5 ml gel) **20361** (10 ml gel)	Binds ≥ 10 mg biotinylated-BSA per ml of resin.
MagnaBind™ Streptavidin Beads	**21344** (5 ml)	Binds 2 µg biotin per ml of beads. Allows magnetic separation. Supporting equipment also available.
Pierce Streptavidin Magnetic Beads	**88816** (1 ml) **88817** (5 ml)	Binds 550 µg biotinylated IgG/ ml beads
Pierce Streptavidin Coated Plates	**15118-15122, 15124-15126** (various 96-well plate packages) **15405-15407** (various 384-well plate packages)	Binds 5 pmoles biotin per well. Large variety of plate formats. Custom-made plates available upon request.
Pierce Streptavidin High Binding Capacity Coated Plates	**15500-15503** (various 96-well plate packages) **15504-15506** (various 384-well plate packages)	Binds 60 pmoles biotin per well, large variety of plate formats. Custom-made plates available upon request.
AminoLink Plus Immobilization Kit	**44894**	Binds primary amines. Standard buffer system and 5 x 2 ml columns included.
Pierce Maleic Anhydride Activated Polystyrene Plates	**15100, 15102, 15110, 15112** (various 96-well plate packages)	Binds primary amines. Custom-made plates available upon request.

References

Kadonaga, J. and Tjian, R. (1986). *Proc. Natl. Acad. Sci. USA* **83**, 5889-5893.

Kneale, G. (1994). *Methods in Molecular Biology,* Volume **30***: DNA-Protein Interactions: Principles and Protocols.*

Thermo Scientific Pierce Affinity Supports for Capturing Protein:Nucleic Acid Interactions

Immobilized avidin and other biotin-binding supports can be used to isolate protein:nucleic acid complexes in which either the protein or the end-labeled nucleic acid is biotinylated. Several different formats such as solid supports, coated plates, magnetic beads and pull-down assays can be applied to this application.

Immobilized Avidin

Strong biotin interaction creates a nearly irreversible bond.

Immobilized avidin can be used in a variety of applications for the affinity purification of biotinylated macromolecules.

Highlights:

- Hybridization of biotinylated RNA to its complementary DNA and binding to immobilized avidin, with subsequent elution of the single-stranded DNA[1]
- Purification of double-stranded DNA[2]

References
1. Manning, J., *et al.* (1977). *Biochemistry* **16**, 1364-1370.
2. Pellegrini, M., *et al.* (1977). *Nucleic Acids Res.* **4**, 2961-2973.

Ordering Information

Product #	Description	Pkg. Size
20219	**Avidin Agarose Resin** Support: Crosslinked 6% beaded agarose Spacer: None (directly attached) Supplied: 50% aqueous slurry containing 0.02% NaN_3)	5 ml
20362	**Avidin Columns**	5 x 1 ml
20225	**Avidin Agarose Resin**	5 x 5 ml

Immobilized Monomeric Avidin

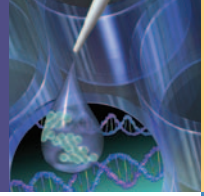
Ideal affinity support for gentle, reversible binding of biotinylated macromolecules.

Highlights:

- Retains biotin specificity with reduced binding affinity (kDa ~10^{-8} M)
- Purifies biotinylated products under mild elution conditions (2 mM free biotin)
- Can be regenerated and reused at least 10 times
- Exhibits little nonspecific binding (3% or less)

Ordering Information

Product #	Description	Pkg. Size
20228	**Monomeric Avidin Agarose**	5 ml
20267	**Monomeric Avidin Agarose**	10 ml
20227	**Monomeric Avidin Kit** Includes: Monomeric Avidin Column BupH Phosphate Buffered Saline Pack (yields 500 ml) Biotin Blocking and Elution Buffer Regeneration Buffer Column Extender	Kit 2 ml 1 pack 200 ml 250 ml
53146	**Monomeric Avidin UltraLink® Resin**	5 ml
29129	**Biotin** *Promotes the gentle elution of biotinylated complex from an immobilized monomeric avidin support.*	1 g



Immobilized NeutrAvidin Supports

Less nonspecific binding makes these exclusive supports well-suited for capturing interacting complexes.

Highlights:

- Carbohydrate-free
- No interaction with cell-surface molecules
- Neutral pI (6.3) eliminates electrostatic interaction
- hat contributes to nonspecific binding

Ordering Information

Product #	Description	Pkg. Size
29200	NeutrAvidin™ Agarose Resin	5 ml
29201	NeutrAvidin Agarose Resin	10 ml
29202	High Capacity NeutrAvidin Agarose Resin	5 ml
29204	High Capacity NeutrAvidin Agarose Resin	10 ml
53150	NeutrAvidin UltraLink Resin Capacity: ~ 12-20 µg of biotin/ml gel	5 ml
53151	NeutrAvidin Plus UltraLink Resin Capacity: ≥ 30 µg of biotin/ml gel	5 ml

Immobilized Streptavidin

High biotin-binding affinity and low nonspecific binding offer advantages for interaction capture.

Highlights:

- Purified recombinant streptavidin
- Stable leach-resistant linkage of streptavidin to the support
- Support: crosslinked 6% beaded agarose
- Capacity: approx. 1-3 mg biotinylated BSA/ml gel

Ordering Information

Product #	Description	Pkg. Size
20347	Streptavidin Agarose Resin	2 ml
20349	Streptavidin Agarose Resin	5 ml
20353	Streptavidin Agarose Resin	10 ml
20357	High Capacity Streptavidin Agarose Resin	2 ml
20359	High Capacity Streptavidin Agarose Resin	5 ml
20361	High Capacity Streptavidin Agarose Resin	10 ml
20351	Streptavidin Columns	5 x 1 ml

UltraLink Immobilized Streptavidin

A high-performance support offering faster flow rates and overall superior performance in affinity applications.

If using immobilized streptavidin for purifying proteins that bind to a biotinylated ligand (DNA or peptides), UltraLink Products are recommended. The Thermo Scientific UltraLink support comes in a "Plus" version, with twice the amount of streptavidin loaded per ml of gel.

Application:

- Recovery of single-stranded DNA for dideoxy sequencing[1]

References
1. Mitchell, L.G. and Merril, C.R. (1989). *Anal. Biochem.* **178**, 239-242.

Ordering Information

Product #	Description	Pkg. Size
53113	Streptavidin UltraLink® Resin Capacity: ≥ 2 mg of biotinylated BSA/ml gel	2 ml
53114	Streptavidin UltraLink Resin	5 ml
53116	Streptavidin Plus UltraLink Resin Capacity: ≥ 4 mg of biotinylated BSA/ml gel	2 ml
53117	Streptavidin Plus UltraLink Resin	5 ml

Ordering Information

Product #	Description	Pkg. Size
87741	High Capacity NeutrAvidin Chromatography Cartridges Support: Crosslinked 6% beaded agarose Capacity: > 75 µg biotinylated p-NPE/ml resin (> 8 mg biotinylated BSA/ml resin)	2 x 1 ml
87742	High Capacity NeutrAvidin Chromatography Cartridge Support: Crosslinked 6% beaded agarose Capacity: > 75 µg biotinylated p-NPE/ml resin (> 8 mg biotinylated BSA/ml resin)	1 x 5 ml
87739	High Capacity Streptavidin Chromatography Cartridges Support: Crosslinked 6% beaded agarose Capacity: > 10 mg biotinylated BSA/ml of resin	2 x 1 ml
87740	High Capacity Streptavidin Chromatography Cartridge Support: Crosslinked 6% beaded agarose Capacity: > 10 mg biotinylated BSA/ml of resin	1 x 5 ml

Thermo Scientific Pierce Coated Plates for Capturing Protein:Nucleic Acid Interactions

Biotin-binding plates offer a convenient platform for designing assays to detect biotinylated molecules in complex samples. These plates are coated with either streptavidin or NeutrAvidin biotin-binding protein, and are pre-blocked to prevent nonspecific binding. We offer three types of biotin-binding plates: Standard Binding Capacity (SBC), High Binding Capacity (HBC) and High Sensitivity (HS) plates. Additionally, these plates are available in different sizes (8-well strips, 96-well and 384-well) and different colors (clear, black or white). Choose a plate with the right specifications to fit your assay's needs.

Comparison of characteristics of Thermo Scientific Pierce Coated Plates.

	High Sensitivity (HS)	High Binding Capacity (HBC)	Standard Binding Capacity (SBC)
Application	Detect low concentrations of biotinylated molecules	Detect high concentrations of biotinylated molecules	General ELISA screening applications
Biotinylated Molecule Minimum Size	> 26 kDa	> 8 kDa	> 8 kDa
Detection Range ** Streptavidin Plates	5-300 ng/ml	62-10,000 ng/ml	31-1,250 ng/ml
Detection Range NeutrAvidin Plates**	5-125 ng/ml	15-2,500 ng/ml	15-300 ng/ml

*** Determined using Thermo Scientific QuantaBlu Fluorogenic Peroxidase Substrate.*

Plate Characteristics for Standard Capacity Biotin-Binding Plates.

Biotin-Binding Protein	Plate Type	Coat Volume*	Block Volume*	Approximate Binding Capacity
NeutrAvidin	96-Well, 8-Well Strip	100 µl/well	200 µl/well	~15 pmol biotin/well
	384-Well	50 µl/well	100 µl/well	~10 pmol biotin/well
Streptavidin	96-Well, 8-Well Strip	100 µl/well	200 µl/well	~5 pmol biotin/well
	384-Well	50 µl/well	100 µl/well	~4 pmol biotin/well

** The coating and blocking volumes give here are typical. Additional variations are also available.*

NeutrAvidin Coated Polystryene Plates (SBC)

The high affinity of avidin for biotin, without the nonspecific binding problems.

Highlights:

- Lowest nonspecific binding properties of all biotin-binding proteins
- NeutrAvidin Biotin-Binding Protein has no carbohydrate and an isoelectric point of 6.3
- Pre-blocked
- Binding capacity: 15 pmoles of biotin/100 µl coat volume

Ordering Information

Product #	Description	Pkg. Size
15123	NeutrAvidin Coated 96-Well Plates with Blocker™ BSA (Clear)	5 plates
15129	NeutrAvidin Coated 96-Well Plates with SuperBlock® Blocking Buffer (Clear)	5 plates
15128	NeutrAvidin Coated Strip Plates with Blocker BSA (Clear)	5 plates
15127	NeutrAvidin Coated Strip Plates with SuperBlock Blocking Buffer (Clear)	5 plates

To view the complete line of NeutrAvidin Coated Plates, visit www.thermoscientific.com/pierce.

Streptavidin Coated Polystyrene Plates (SBC)

The specific binding affinity of streptavidin for biotin – in a microplate.

Highlights:

- Gentle immobilization of biotinylated complexes
- Low nonspecific binding
- Pre-blocked
- Binding capacity: 5 pmoles of biotin/100 µl coat volume (96-well plates)

Ordering Information

Product #	Description	Pkg. Size
15120	Streptavidin Coated Polystyrene Strip Plates with SuperBlock Blocking Buffer (Clear)	5 plates
15124	Streptavidin Coated 96-Well Plates with SuperBlock Blocking Buffer (Clear)	5 plates
15125	Streptavidin Coated 96-Well Plates with Blocker BSA (Clear)	5 plates
15126	Streptavidin Coated 96-Well Plates with SuperBlock Blocking Buffer (Clear)	25 plates

To view the complete line of Streptavidin Coated Plates, visit www.thermoscientific.com/pierce.

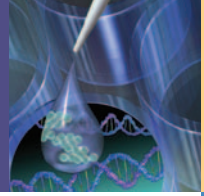


Plate Characteristics for High Binding Capacity Biotin-Binding Plates.

Biotin-Binding Protein	Plate Type	Coat Volume	Block Volume (SuperBlock Blocking Buffer)	Approximate Binding Capacity
NeutrAvidin	96-Well, 8-Well Strip	100 µl/well	200 µl/well	~60 pmol biotin/well
	384-Well	50 µl/well	100 µl/well	~35 pmol biotin/well
Streptavidin	96-Well, 8-Well Strip	100 µl/well	200 µl/well	~125 pmol biotin/well
	384-Well	50 µl/well	100 µl/well	~60 pmol biotin/well

NeutrAvidin High Binding Capacity (HBC) Coated Plates

Unique technology for improved assay precision.

Highlights:

- Unique plate-coating technology for high binding capacity
- Improved sensitivity
- Broader dynamic range
- Pre-blocked
- Flexble assay formats (see www.thermoscientific.com/pierce for complete listing)

Ordering Information

Product #	Description	Pkg. Size
15507	NeutrAvidin Coated Plates (HBC), 96-well Plates with SuperBlock Blocking Buffer (Clear)	5 plates
15508	NeutrAvidin Coated Plates (HBC), 8-well Strips, with SuperBlock Blocking Buffer (Clear)	5 plates
15509	NeutrAvidin Coated Plates (HBC), 96-well Plates with SuperBlock Blocking Buffer (White)	5 plates
15510	NeutrAvidin Coated Plates (HBC), 96-well Plates with SuperBlock Blocking Buffer (Black)	5 plates

Streptavidin HBC Coated Plates

Special coating technology results in four to five times the typical binding capacity.

Highlights:

- Increased sensitivity of complex detection
- Broader dynamic range
- Pre-blocked
- Flexible assay formats (see www.thermoscientific.com/pierce for complete listing)
- High-binding capacity (HBC)

Ordering Information

Product #	Description	Pkg. Size
15500	Streptavidin Coated Plates (HBC), 96-well Plates with SuperBlock Blocking Buffer (Clear)	5 plates
15501	Streptavidin Coated Plates (HBC), 8-well strips with SuperBlock Blocking Buffer (Clear)	5 plates
15502	Streptavidin Coated Plates (HBC), 96-well Plates with SuperBlock Blocking Buffer (White)	5 plates
15503	Streptavidin Coated Plates (HBC), 96-well Plates with SuperBlock Blocking Buffer (Black)	5 plates

Streptavidin and NeutrAvidin Coated Plates – High Sensitivity

High sensitivity biotin-binding plates for low-level target detection.

Highlights:

- Sensitive – detect down to 5 ng/ml of biotinylated IgG
- Specific – low background with high signal-to-noise ratios
- Versatile – use either fluorescence, chemiluminescence or colorimetric detection
- Robust – broad dynamic range
- Ready to use – plates are supplied pre-blocked to save time

Ordering Information

Product #	Description	Pkg. Size
15520	Streptavidin Coated Plates (High Sensitivity), 8-well Strips with Blocker BSA (Clear)	5 plates
15525	Streptavidin Coated Plates (High Sensitivity), 8-well Strips with Blocker BSA (Black)	5 plates
15530	NeutrAvidin Coated Plates (High Sensitivity), 8-well Strips with Blocker BSA (Clear)	5 plates
15535	NeutrAvidin Coated Plates (High Sensitivity), 8-well Strips with Blocker BSA (Black)	5 plates

Magnetic Beads for Capture of Interacting Complexes

Thermo Scientific MagnaBind Streptavidin Coated Magnetic Beads

A convenient method for isolating biomolecules using affinity binding, while retaining biological activity.

Highlights:

- Fast capture and separation of complexes
- Beads respond well to weak magnetic fields
- 1-4 µm diameter
- 1 x 10^8 particles/mg

Ordering Information

Product #	Description	Pkg. Size
21344	MagnaBind Streptavidin Beads	5 ml
21357	Magnet for 1.5 ml Microcentrifuge Tube	1 magnet
21359	Magnet for 6 Microcentrifuge Tubes	1 magnet

Thermo Scientific Pierce Streptavidin Magnetic Beads

Accelerate magnetic purification of biotinylated molecules from samples.

Highlights:

- High-performance, non-aggregating, pre-blocked, iron oxide, superparamagnetic microparticles
- Stable immobilization chemistry
- Nearly three times higher binding capacity
- Low non-specific binding

Ordering Information

Product #	Description	Pkg. Size
88816	Pierce Streptavidin Magnetic Beads	1 ml
88817	Pierce Streptavidin Magnetic Beads	5 ml

Pull-Down Assay for Capture of Interacting Complexes

Thermo Scientific Pierce Pull-Down Biotinylated-Protein Interaction Kit

Pull-down a binding partner with the Thermo Scientific Pierce Biotinylated-Protein Interaction Kit.

See the complete description of this kit in the Pull-Down Assays segment of this handbook, page 19.

Ordering Information

Product #	Description	Pkg. Size
21115	Pull-Down Biotinylated-Protein Interaction Kit	Kit

Chemiluminescent Detection in Polystyrene Plates

Thermo Scientific SuperSignal ELISA Pico and Femto Chemiluminescent Substrates

Experience the same sensitivity in your luminometer that you've come to expect from all SuperSignal Products.

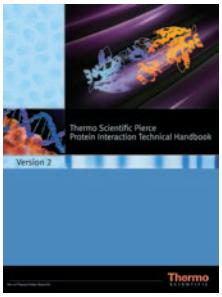
See the complete description of this product at www.thermoscientific.com/pierce.

Ordering Information

Product #	Description	Pkg. Size
37070	SuperSignal ELISA Pico Chemiluminescent Substrate	100 ml
	Includes: Luminol/Enhancer	50 ml
	Stable Peroxide Buffer	50 ml
37075	SuperSignal ELISA Femto Maximum Sensitivity Substrate	100 ml
	Includes: Luminol/Enhancer	50 ml
	Stable Peroxide Buffer	50 ml

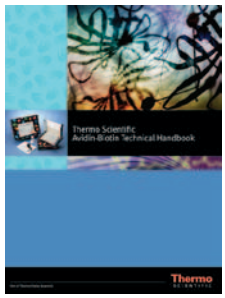
Handbooks

Protein Interaction Technical Handbook



The study of protein interactions is vital for understanding protein function within the cell. This handbook provides background, helpful hints and troubleshooting for methods used to study these interactions, including IP and co-IP, pull-downs, far-Western blotting, and crosslinking.
(Product # 1601645)

Avidin-Biotin Technical Handbook



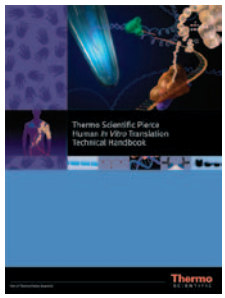
This guide brings together everything needed to biotinylate cell-surface proteins, purify a biotinylated target, detect a biotinylated antibody and perform many other applications. It includes dozens of references along with protocols, troubleshooting tips, selection guides and a complete listing of available tools.
(Product # 1601675)

Crosslinker Technical Handbook

For the complete Thermo Scientific Pierce Crosslinker Products offering, including a broad selection of heterobifunctional-photo-reactive crosslinking reagents, request a free copy of our Crosslinker Technical Handbook (Product # 1601673).

An online Crosslinker Selection Guide can also be found at www.thermoscientific.com/pierce.

Human *In Vitro* Translation Technical Handbook



This 52-page technical handbook covers the basic types of *in vitro* translation systems and delves deeper into the newer human-based cell-free translations systems. The handbook also looks at the advantages of the human systems for glycoprotein production. The guide finishes with numerous applications of *in vitro* synthesized proteins, including the study of protein interactions, protein purification and protein labeling.
(Product # 1601865)

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