Protein–nucleic acid interactions

Protein extraction | DNA electrophoretic mobility shift assay (EMSA) | Chromatin immunoprecipitation (ChIP) | RNA EMSA | RNA pull-down | Biotin and desthiobiotin nucleic acid labeling

ThermoFisher SCIENTIFIC

Introduction

Proteins and nucleic acids do not operate within biological systems as independent entities. Protein–nucleic acid interactions are involved in multiple processes essential to normal cell function. Disruption of such interactions leads to serious and often profound consequences within the system.

Protein–nucleic acid interactions are integrated into several key cellular processes. These processes include transcription, translation, and regulation of gene expression, recognition, replication, recombination, repair, nucleic acid packaging, chromatin remodeling, and the formation of cellular machinery such as ribosomes. The role of DNA as the genetic repository of information requires interaction with proteins for the extraction of this information for timely utilization within the cell.

The common property of nucleic acid–binding proteins is their ability to recognize and manipulate DNA/RNA structures. Depending on the nature of the complex, proteins associate with nucleic acids in the major or minor helical grooves in a sequence-specific or secondary structure–dependent manner, often inducing drastic structural changes in the nucleic acid. Defining sequence-specific interactions can aid in the development of high-affinity aptamers, which may be used as purification tools for DNA- or RNA-binding proteins. Sequence-specific interactions also provide critical information in the study of gene regulation and drug discovery research.

Table 1. Overview of Thermo Scientific™ kits used to study protein–nucleic acid interactions.

Product	LightShift™ EMSA kit	MAGnify™ Chromatin IP kit	LightShift™ RNA EMSA kit	Pierce™ Magnetic RNA-Protein Pull-Down Kit
Technique	Electrophoretic mobility shift assay (EMSA)	Chromatin immunoprecipitation (ChIP)	RNA EMSA	RNA immunoprecipitation (RIP)
Target	Protein–DNA interaction	Protein–DNA interaction	Protein–RNA interaction	Protein–RNA interaction
Interaction conditions	*In vitro*	*In vivo*	*In vitro*	*In vitro*
Nucleic acid labeling method	Biotin	NA	Biotin	Desthiobiotin (included in kit)
Base bead	NA	Dynabeads™ Protein A/G	NA	Pierce™ nucleic acid–compatible streptavidin magnetic beads
Detection method	Membrane-based, using streptavidin-HRP chemiluminescence	qPCR or next-generation sequencing (NGS)	Membrane-based, using streptavidin-HRP chemiluminescence	Western blot or mass spectrometry of intact complexes
Goal	Characterize protein binding to DNA sequence	Monitor transcription regulation (qPCR) or identify target sequence (NGS)	Characterize protein binding to RNA sequence	Enrichment of low-abundance targets
Preparation and sample processing time	4.5–5 hr	5.5 hr	7–8 hr	3 hr (hands-on time only)

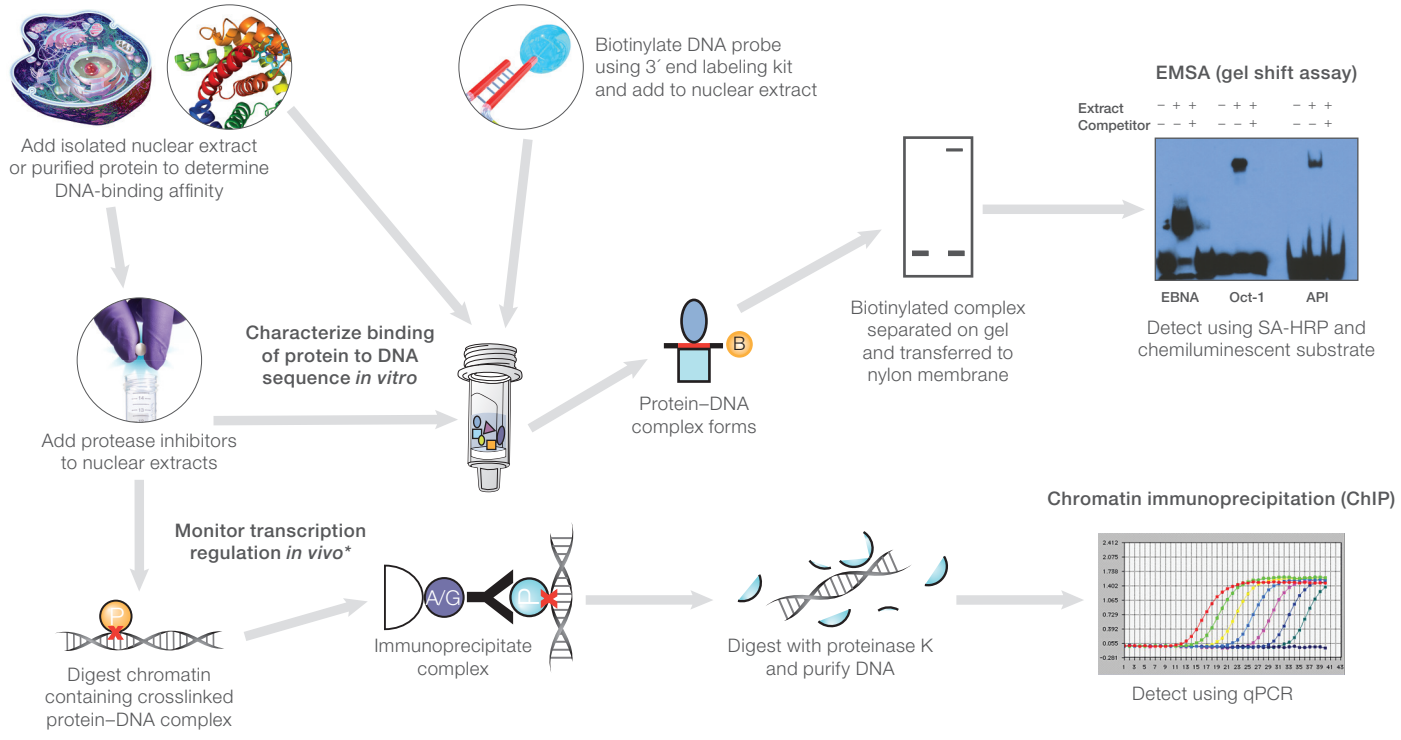
Learn more at **thermofisher.com/protein-nucleic-interactions**

Protein–DNA interaction studies

A major function of protein–DNA interactions is to manage the extensive length of the genetic material contained in each cell. Chromosomes, regulated by complex protein–DNA interactions, have evolved physical material of the DNA into condensed structures that impact the cell's access to genetic information. Chromosomal structure also plays a role in transcription. During chromatin remodeling, selective portions of a gene's regulatory region unravel, making the DNA available for gene transcription, or become tightly packaged, completely silencing the transcription of the genes.

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 signal 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.

A number of laboratory techniques have been developed to study these complex interactions—each with a unique history with varying utility and distinct strengths and weaknesses. These include DNA EMSA or gel-shift assay, transcription factor assays, ChIP assays, and NGS assays. Specifically, the EMSA and ChIP assays will be described in this section.



* DNA is crosslinked prior to cell fractionation

Figure 1. Overview of three protein–DNA interaction workflows.

NE-PER Nuclear and Cytoplasmic Extraction Kit

Fast, easy-to-use kit for isolating nuclear extracts

The Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Kit enables efficient cell lysis, and extraction of cytoplasmic and nuclear protein fractions separately in less than 2 hours.

The NE-PER kit is a nuclear protein extraction method that 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 (gDNA) and mRNA. Once desalted or diluted, the isolated proteins can be used to perform immunoassays and protein interaction experiments, such as mobility shift assays (EMSA), co-immunoprecipitation (Co-IP) and pull-down assays.

Highlights:

- **Fast**—obtain nuclear and cytoplasmic fractions of soluble proteins in typically less than 2 hours
- **Proven**—the NE-PER kit is referenced in more than 950 distinct publications
- **Versatile**—nuclear protein extraction from either cultured cells or tissues (intended for fresh samples only)
- **Scalable**—two kit sizes for producing extracts from cells and tissues
- **Convenient**—simple instructions do not require gradient ultracentrifugation
- **Compatible**—use for downstream assays, including western blotting, gel-shift assays, protein assays, reporter gene assays, enzyme activity assays, and others
- **Robust**—verified in heart, lung, kidney, and liver tissue, and HeLa, NIH 3T3, A549, C6, COS-7, and Hepa cultured mammalian cells

The preparation of good nuclear protein extracts is central to the success of many gene regulation studies. Nuclear extracts are used instead of whole-cell lysates for the following reasons: first, many experiments in the area of gene regulation are adversely affected by cellular components present in whole-cell lysates, and second, the concentration of the nuclear protein of interest is diluted by the vast array of cytoplasmic proteins present in whole-cell extracts. Finally, whole-cell lysates are complicated by the presence of gDNA and mRNA.

A variety of methods exist to isolate nuclei and prepare nuclear protein extracts. However, 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 Nuclear and Cytoplasmic Extraction Kit enables a stepwise lysis of cells that generates both functional cytoplasmic and nuclear protein fractions, typically in less than 2 hours.

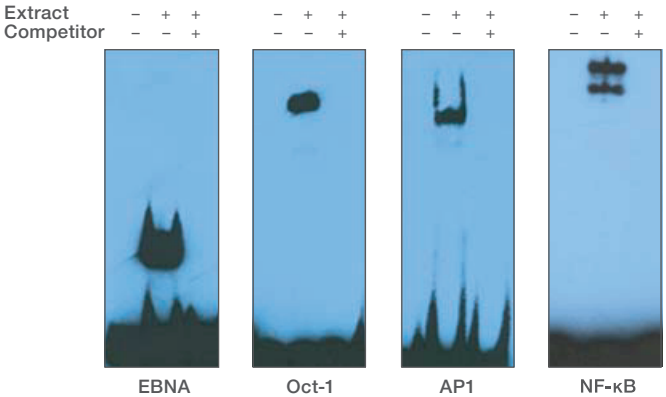


Figure 2. Chemiluminescent EMSA of four different DNA–protein complexes. DNA-binding reactions were performed using 20 fmol of biotin-labeled DNA duplex (1 biotin per strand) and 2 µL (6.8 µg total protein) of NE-PER Nuclear Extract prepared from HeLa cells. For reactions containing specific competitor DNA, a 200-fold molar excess of unlabeled specific duplex was used.

Protease and phosphatase inhibitors

Broad-spectrum liquid cocktails and tablets for complete protein protection

Protease and phosphatase inhibitor cocktails and tablets are ideal for the protection of proteins during extraction or lysate preparation from primary cells, cultured mammalian cells, animal tissues, plant tissues, yeast cells, or bacterial cells. Formulations are packaged in multiple sizes, and EDTA-free versions are available for divalent cation–sensitive assays. The Thermo Scientific™ Pierce™ inhibitor tablets have been reformulated to dissolve quickly into a clear solution, and are fully compatible with all Thermo Scientific™ Pierce™ protein assays.

Highlights:

- **Convenient**—ready-to-use, fully disclosed, broad-spectrum formulations available as either liquid cocktails or tablets, in multiple pack sizes, and with a minimum 1-year shelf life
- **Complete protection**—combined cocktails available with all-in-one formulations containing both protease and phosphatase inhibitors
- **Compatible**—use directly with Thermo Scientific™ Pierce™ Cell Lysis Buffers or other commercial or homemade detergent-based lysis reagents

Table 2. Components present in Thermo Scientific™ Halt™ Protease Inhibitor Cocktails and Pierce™ Protease and Phosphatase Inhibitor Tablets.

Inhibitor component	Target (mechanism)	Protease liquid cocktails and tablets	Phosphatase liquid cocktails and tablets	Combined protease and phosphatase liquid cocktails and tablets
AEBSF·HCl	Serine proteases (irreversible)	●		
Aprotinin	Serine protease (reversible)	●		●
Bestatin	Aminopeptidase (reversible)	●		●
E-64	Cysteine (irreversible)	●		●
Leupeptin	Serine and cysteine proteases (reversible)	●		●
Pepstatin	Aspartic acid proteases (reversible)	●		
EDTA*	Metalloproteases (reversible)	●		●
Sodium fluoride	Serine/threonine and acidic phosphatases		●	●
Sodium orthovanadate	Tyrosine and alkaline phosphatases		●	●
β-Glycero-phosphate	Serine/threonine phosphatase		●	●
Sodium pyrophosphate	Serine/threonine phosphatase		●	●

* EDTA not in EDTA-free formulations.

Pierce Biotin 3′ End DNA Labeling Kit

Complete kit for labeling the 3′ end of DNA with biotin

The Thermo Scientific™ Pierce™ Biotin 3′ End DNA Labeling Kit is for tagging single-stranded DNA primers with biotin for use in nonradioactive EMSA and other nucleic acid detection methods. The DNA biotinylation procedure uses terminal deoxynucleotidyl transferase (TdT) to catalyze nontemplate-directed nucleotide incorporation onto the 3′-OH end of DNA. 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.

Highlights:

- Non-isotopic labeling eliminates the hassle of waste that is hazardous or difficult to dispose of
- 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 1 year
- 30-minute labeling procedure is fast and efficient

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 EMSA, northern or southern blots, colony hybridizations, or *in situ* hybridizations.

Tech tip

Many methods can be used to anneal complementary strands of nucleic acids. In each case, the goal is to denature the complementary strands to remove any secondary structure and then allow the strands to hybridize. See Tech Tip #45: “Anneal complementary pairs of oligonucleotides”

Find these and other helpful tech tips at
thermofisher.com/protein-nucleic-interactions

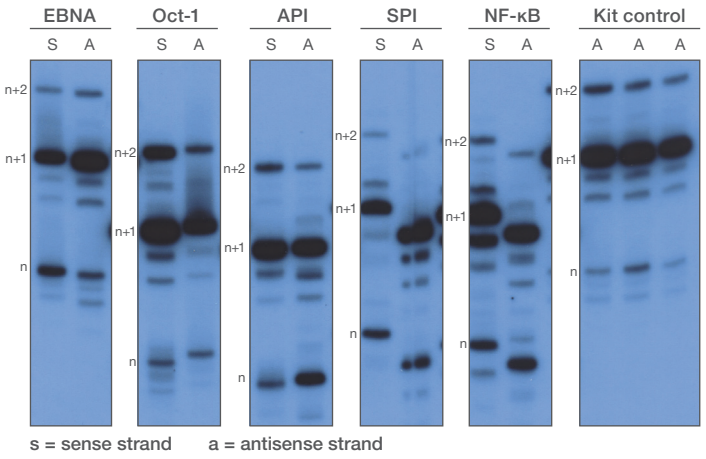


Figure 3. Sequencing gel analysis of labeling efficiency. Ten different oligos (ranging in size from 21–25 nt) were labeled using the Pierce Biotin 3′ End DNA Labeling Kit. The products from the TdT reaction were then radiolabeled using T4 polynucleotide kinase (PNK) and [γ^{32}P]ATP. The PNK reactions 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 1 or 2 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.

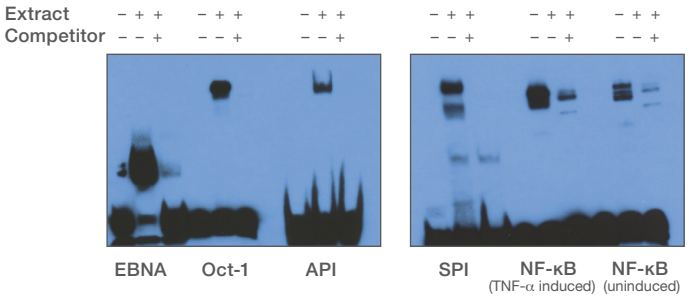
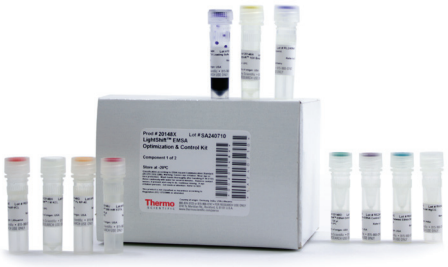


Figure 4. EMSA results using 3′ biotin-labeled DNA duplexes. The sense and antisense strands were labeled using the Biotin 3′ End DNA Labeling Kit and hybridized for 4 hr at room temperature to form duplexes containing the binding sites for the indicated transcription factors. Gel shift assays were performed 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 NE-PER Nuclear and Cytoplasmic Extraction Reagents (Cat. No. 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 TNF-α 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.

LightShift Chemiluminescent DNA EMSA Kit

Robust kit for identifying and characterizing protein–DNA binding interactions



The LightShift Chemiluminescent DNA EMSA Kit is an extraordinarily robust and sensitive system for performing 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.

Highlights:

- Includes Epstein-Barr virus nuclear antigen (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-based methods
- Compatible with previously established binding conditions for popular DNA–protein interactions

The principle for our DNA EMSA detection kit is similar to that of a western blot. Biotin 3′ 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 (Cat. No. 89818). Nuclear, cytosolic, or whole-cell protein extracts may be obtained by a variety of methods, including the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Cat. No. 78833).

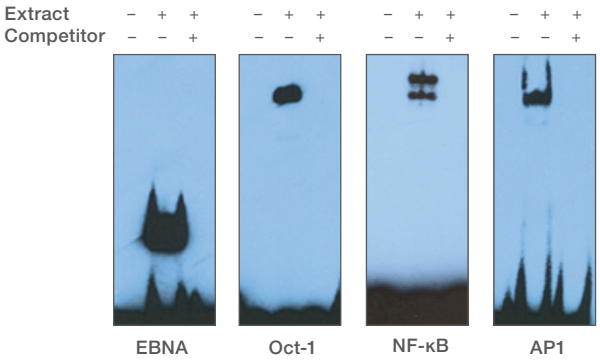


Figure 5. Chemiluminescent DNA EMSA of four different DNA–protein complexes. Biotin-labeled target duplexes ranged in size from 21 to 25 bp. The EBNA reactions were supplemented with 2.5% glycerol and 0.05% NP-40, and the AP1 reactions were supplemented with 10% glycerol. The Oct-1, AP1, and NF-κB transcription factors were obtained from 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. The exposure times to X-ray film for each system ranged from 2 min for EBNA, Oct-1, and AP1, and 5 min for NF-κB.

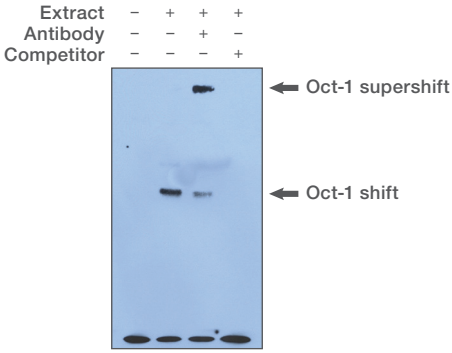


Figure 6. The LightShift EMSA kit has been shown to work with supershifts. LightShift EMSA using a 22 bp Oct-1 specific duplex and a HeLa cell extract is shown. Rabbit anti–Oct-1 antibody (1 µg) was added to the binding reaction last and incubated at room temperature for 20 min before loading the native polyacrylamide gel. Film exposure time was 2 min.

Learn more at **thermofisher.com/emsa**

MAGnify Chromatin Immunoprecipitation System

Convenient, complete magnetic kit for ChIP assays

The Invitrogen™ MAGnify™ Chromatin Immunoprecipitation System provides a streamlined, optimized assay for the enrichment of chromatin/protein complexes and DNA recovery using magnetic bead capture technology. The isolated DNA is ready for downstream analysis by methods such as PCR- or qPCR-based assays, or massive parallel DNA sequencing.

Highlights:

- **Complete**—kit includes all components needed for ChIP-based workflows
- **Fast**—prepare sample in less than 6 hours
- **Convenient**—magnetic bead format allows easy sample processing
- **Flexible**—immunoprecipitated DNA is compatible with PCR- or qPCR-based assays, or NGS

Chromatin immunoprecipitation (ChIP) is a powerful technique for studying the association of certain proteins with specific regions of the genome. These sequence-specific DNA-binding proteins are believed to play a role in such cellular processes as DNA replication, recombination, repair, and segregation; chromosomal stability; cell-cycle progression; and epigenetic silencing. In a standard ChIP assay, a cell is fixed via formaldehyde treatment; and the chromatin is sheared and immunoprecipitated via a highly specific antibody. The researcher then analyzes the DNA to identify the genomic regions where the chromatin-associated proteins bind to the chromatin *in vivo*. This kit enables researchers to start with lower sample amounts than in traditional ChIP workflows, thereby preserving precious samples, and the protocol can be completed in a single day, compared with 2–3 days for a traditional ChIP assay. The kit can be used with our suite of ChIP-verified antibodies, and is also complementary with Applied Biosystems™ MethylCode™ and NCode™ products for downstream epigenetics research. The Dynabeads Protein A and Protein G magnetic beads can be purchased separately.

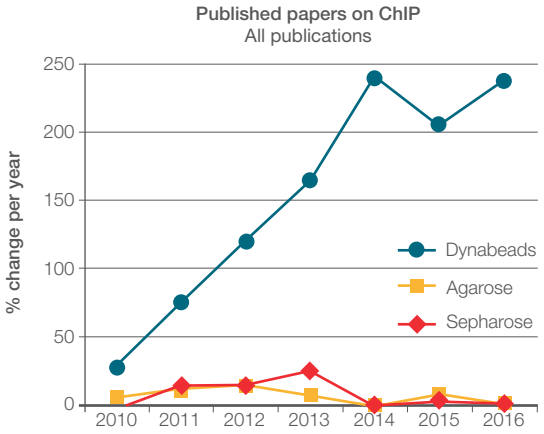


Figure 7. ChIP publication growth (Dynabeads magnetic beads compared to nonmagnetic methods). (Source: January 2017 Google Scholar)

With the advent of NGS, ChIP has become even more powerful—researchers can now get a snapshot of not only specific protein–DNA interactions in a few regions but also genome-wide interactions, by adapting purified ChIP DNA for NGS, called ChIP-sequencing (ChIP-Seq).

When using the MAGnify system, cells or tissues are treated with formaldehyde to generate protein–protein and protein–DNA crosslinks between molecules in close proximity within the chromatin complex. The cells are then lysed, and the chromatin is released from the nuclei and sheared by sonication to reduce the average DNA fragment size to 200–500 bp for analysis by quantitative real-time PCR (qPCR) or 100–300 bp for analysis by massive parallel DNA sequencing. The crosslinked protein of interest is then immunoprecipitated, using a specific ChIP-qualified antibody conjugated to Invitrogen™ Dynabeads™ Protein A/G magnetic beads. The formaldehyde crosslinking is reversed by heat treatment, and the DNA associated with that protein is purified. The DNA is now ready for downstream analyses such as end-point PCR or qPCR, genome-wide analyses using promoter-tiling arrays, or NGS. In PCR/qPCR analysis, primers are designed to span the desired DNA sequence of interest, and the data demonstrates whether the specific protein of interest is associated *in vivo* with that DNA region.

Table 3. Comparison to a conventional ChIP protocol.

Workflow step	MAGnify ChIP timeline	Conventional ChIP timeline
Pre-clearing	NA	1–2 hr
Antibody/chromatin incubation	2 hr	Overnight
Bead pulldown	1 hr	2 hr
Washes	30 min (2 buffers)	1–3 hr (4 buffers)
Reverse crosslinking	1.5 hr	Overnight
Proteinase K digestion		2 hr
DNA elution from beads		15–30 min
DNA purification		2 hr–overnight
Average time	**5 hr**	**36–48 hr**

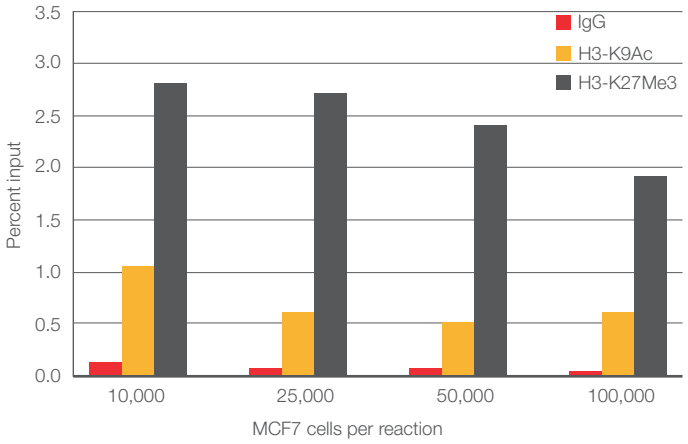


Figure 8. Titration of cell number with MAGnify ChIP kit. Sheared chromatin from MCF7 cells was prepared from 10^6 cells in 50 µL lysis buffer and diluted to indicated number of cells per ChIP according to the MAGnify ChIP protocol. 1 µg of each antibody (IgG, included in kit; H3-K27Me3, Cat. No. 49-1014; H3-K9Ac, Cat. No. 49-1009) were used for each ChIP experiment. 1% of the sonicated chromatin was set aside as input control. Optimized qPCR primers were used to amplify the SAT2 locus (Cat. No. 49-2026). Data are graphed as percent input.

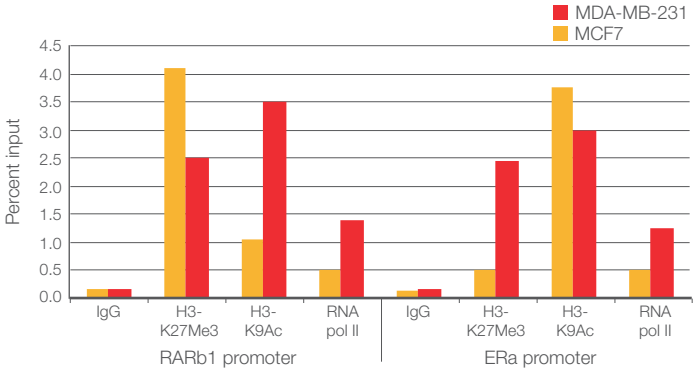


Figure 9. 10,000 cell input with MAGnify ChIP. Sheared chromatin from MCF7 or MDA-MB231 cells was prepared from 10^6 cells in 50 µL lysis buffer and diluted to 10,000 cells per ChIP, according to the MAGnify ChIP protocol. 1 µg of antibody (IgG, included in kit; H3-K27Me3, Cat. No. 49-1014; H3-K9Ac, Cat. No. 49-1009) or 3 µL RNA pol II (Cat. No. 49-1033) were used for each ChIP experiment. 1% of the sonicated chromatin was set aside as input control. Optimized qPCR primers were used to amplify to different loci: RARb1 promoter (Cat. No. 49-2027) or ERa promoter. Data are graphed as percent input.

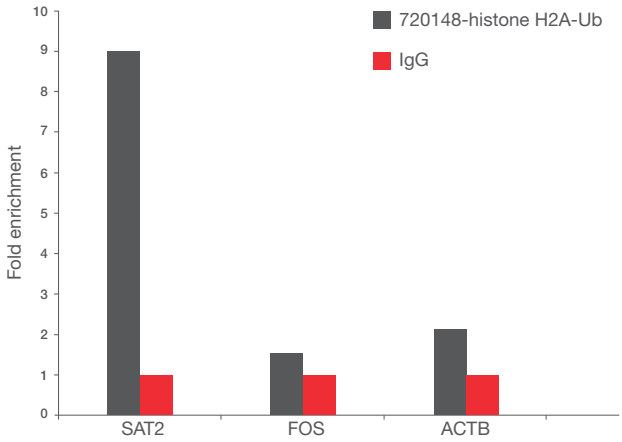


Figure 10. Enrichment of endogenous histone H2A-Ub using anti–histone H2A-Ub rabbit polyclonal antibody. ChIP was performed using Invitrogen™ anti–H2A-Ub rabbit polyclonal antibody (Cat. No. 720148) on sheared chromatin from 2 x 10^6 HeLa cells using the MAGnify Chromatin Immunoprecipitation System (Cat. No. 49-2024). Normal rabbit IgG was used as a negative IP control. The purified DNA was analyzed on the Applied Biosystems™ 7500 Fast Real-Time PCR System (Cat. No. 4351106) with optimized primers for the region of the inactive SAT2 satellite repeat, used as a positive control target, and for promoters of the active cFOS (FOS) and β-actin (ACTB) regions, used as negative control targets. Data are presented as fold enrichment of the antibody signal vs. the negative control IgG, calculated using the comparative C_t method.

Learn more at
thermofisher.com/chip

Protein–RNA interaction studies

Protein–RNA interactions are critical in both the translation of mRNA into protein and in the regulatory roles of noncoding RNA. Many studies have focused on the significance of noncoding RNAs, including the 5´ and 3´ untranslated regions (UTR) of mRNA, small interfering RNA (siRNA), and microRNA (miRNA) families. More recently, some of these small RNAs have been implicated as proto-oncogenes and in various diseases, including cancer. The use of radioactive components, high background and high experimental variability have made studying protein–RNA interactions extremely challenging. The UTR regions of mRNA contain sequence elements that recruit RNA-binding proteins for posttranscriptional 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. 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 noncoding RNAs that initiate posttranscriptional silencing of target mRNA. 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.

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, fluorescence *in situ* hybridization (FISH)/ISH co-localization studies, northwestern analysis and RNase protection assays. RNA EMSA and protein–RNA pull-down assays will be described in this section.

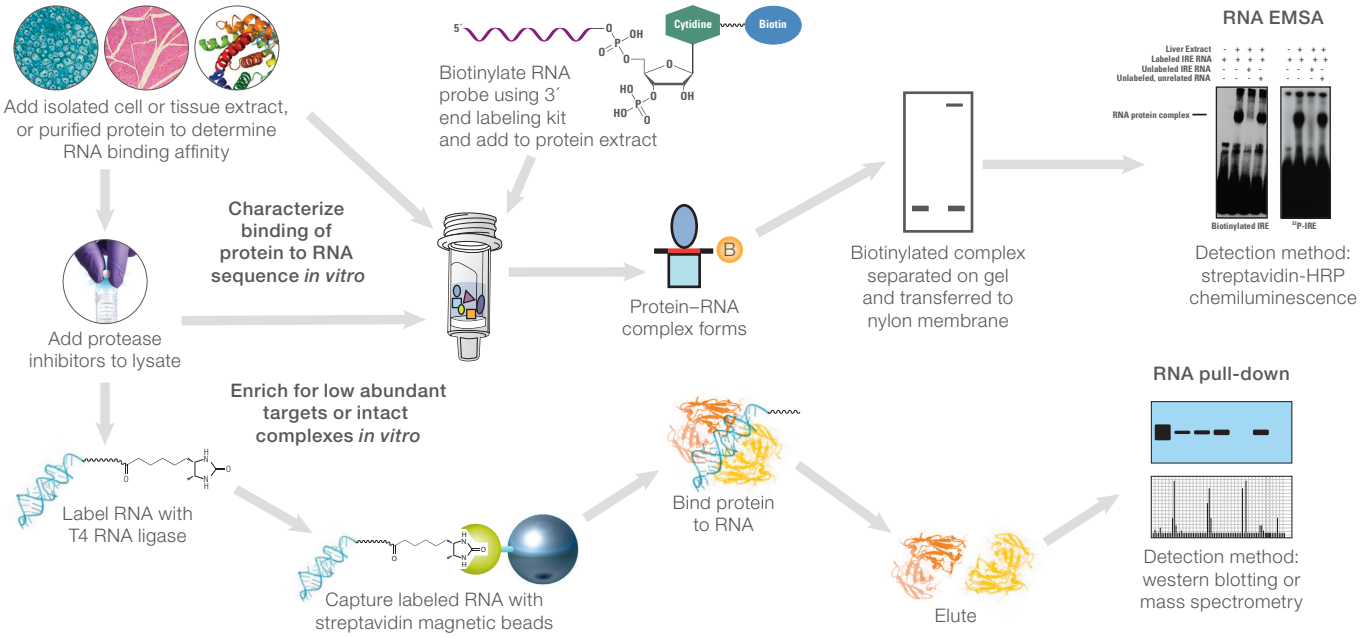


Figure 11. Overview of two protein–RNA interaction workflows.

M-PER Mammalian Protein Extraction Reagents

Gentle formulation designed for total protein extraction from mammalian cells

Thermo Scientific™ M-PER™ Mammalian Protein Extraction Reagent is designed to provide highly efficient total soluble protein extraction from cultured mammalian cells. The M-PER reagent is a nondenaturing detergent formulation that dissolves cell membranes and extracts the total soluble cellular protein in only 5 minutes. The M-PER reagent requires little or no mechanical disruption, does not denature proteins, and is compatible with downstream assays.

Highlights:

- **Gentle**–mild detergent lysis, yielding active protein extracts that are immediately compatible with protein assays, reporter gene assays, western blotting, immunoprecipitation, and affinity purification
- **Easy to use**—amine-free and fully dialyzable formulation enables compatibility with subsequent assay systems
- **Convenient**—lyse adherent cells directly in plate or after harvesting and washing in suspension
- **Robust**—validated for yield and extraction efficiency in primary and cultured mammalian cells

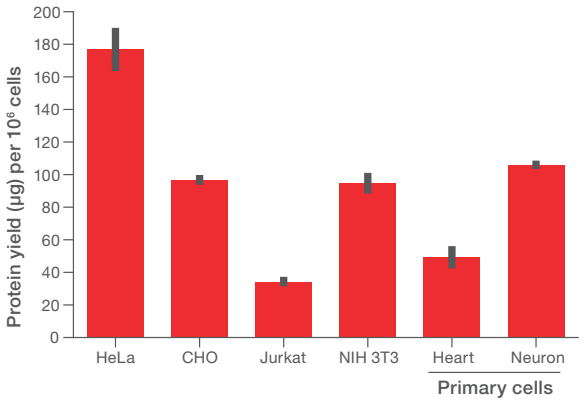


Figure 12. Protein yield from various cell types using M-PER Mammalian Protein Extraction Reagent. Cells were harvested at 85% confluency, washed twice, and collected in ice-cold PBS and counted. For each cell type, 1 x 10^6 cells were pelleted by centrifugation at 2,000 x *g* for 5 min and lysed in 1 mL M-PER reagent for 5 min. The cell lysates were clarified by centrifugation at 14,000 x *g* for 10 min, the supernatant was collected, and the protein concentration (µg/10^6 cells) was determined using the Thermo Scientific™ Pierce™ BCA Protein Assay (Cat. No. 23227).

T-PER Tissue Protein Extraction Reagent

Mild solution designed for total protein extraction from tissue samples

Thermo Scientific™ T-PER™ Tissue Protein Extraction Reagent uses a proprietary detergent in 25 mM bicine, 150 mM sodium chloride (pH 7.6) to maximize the efficiency of protein solubilization from mammalian tissue samples by homogenization. The simple composition of this reagent is compatible with additives such as protease inhibitors, salts, reducing agents and chelating agents, providing versatility for many different sample types and lysis applications. Cell lysates prepared with T-PER reagent are directly compatible with reporter assays (e.g., luciferase, β-galactosidase, chloramphenicol acetyl transferase), protein kinase assays (e.g., PKA, PKC, tyrosine kinase), immunoassays (e.g., western blots, ELISAs, radioimmunoassays (RIAs)) and/or protein purification procedures.

Highlights:

- **Simple procedure**—homogenize tissue sample in 1:20 (w/v) of tissue to T-PER reagent, then centrifuge to pellet cell or tissue debris
- **Easy to use**—mild detergent is dialyzable for quick and easy removal
- **Versatile**—can be used with additional components (e.g., protease inhibitors, salts, reducing agents, chelating agents)
- **Compatible**—the lysate is compatible with standard protein assays such as the Thermo Scientific™ Pierce™ Coomassie Plus (Bradford) Protein Assay (Cat. No. 23236) and the Thermo Scientific™ Pierce™ 600nm Protein Assay (Cat. No. 22660), and may be used for reporter assays, protein kinase assays, immunoassays, ELISAs, western blots, and protein purifications
- **Robust**—verified for yield and extraction efficiency in heart, liver, kidney, lung, and spleen tissues

Pierce RNA 3′ End Biotinylation Kit

A nonradioactive, noninterfering 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 are 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 nonradioactive RNA labeling with minimal interference to the RNA secondary structure.

Highlights:

- **Nonradioactive**—incorporates a biotin label with detection sensitivity comparable to that of radioactivity
- **Fast**—RNA can be labeled typically 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′ End 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 12). The kit also contains an unlabeled 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.

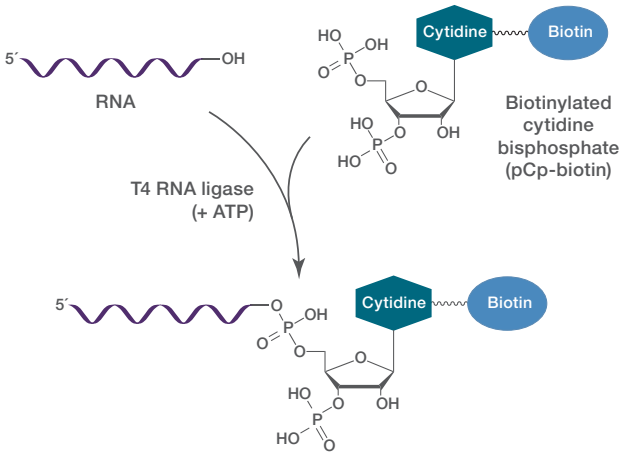


Figure 13. Scheme of T4 RNA ligation reaction. RNA ligation using T4 RNA ligase requires a 3′-OH (from the desired RNA), and a 3′, 5′ nucleotide (bis)phosphate. The Pierce RNA 3′ End Biotinylation Kit contains T4 RNA ligase and a cytidine (bis)phosphate nucleotide with a biotinylated linker for detection.

The kit provides a starting point for the ligation reaction. Typically, the reaction requires 50 pmol of RNA that 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). 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 EMSA (LightShift Chemiluminescent RNA EMSA, Cat. No. 20158), RNA pull-down assays, and miRNA profiling.

Table 4. 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 hr at 16°C	Leibold, et al.
RNA polymerase template	RNA	Synthetic	42	80**	2 hr 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 hr at 16°C	Hall-Pogar, et al.
Let-7 pre-miRNA	Pre-miRNA	*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 min 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 (Cat. No. 89880), and spectroscopy with the Thermo Scientific™ Fluorescent Biotin Quantitation Kit (Cat. No. 46610). Biotin end-labeled IRE and RNA polymerase template RNAs have sensitivity comparable to a synthetically biotinylated RNA (Figure 13). 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).

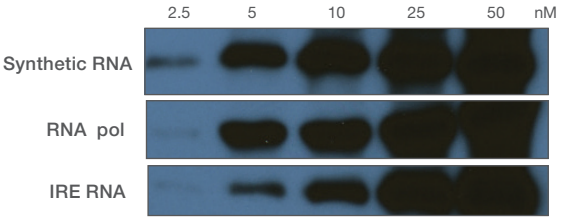


Figure 14. End-labeled RNAs have sensitivity similar to synthetically labeled RNA. Two end-labeled RNA probes made with the Pierce 3′ End Biotinylation Kit (RNA pol and IRE RNA) and a synthetic biotinylated RNA probe (synthetic RNA) were electrophoresed on a 10% acrylamide/ 8 M urea gel, transferred to a nylon membrane, UV crosslinked, and detected using the Chemiluminescent Nucleic Acid Detection Module (Cat. No. 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 *in vitro*–transcribed using T7 RNA polymerase. The different RNA used in this study had diverse secondary structure and lengths. Each of the four synthetic and both of the *in vitro*–transcribed RNAs had ligation efficiencies of >70%, demonstrating that the kit provides flexible and produces efficient biotinylation for RNA of different length, secondary structure, and source.

Once labeled, a biotinylated RNA probe can be used as a 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), and detected using a chemiluminescent substrate.

Tech tip

The 5′ phosphate group of oligonucleotides, DNA and RNA can be conjugated to primary amine-containing molecules using the carbodiimide crosslinker EDC and imidazole. The strategy can be adapted in a number of ways to directly or indirectly modify, label (e.g. biotinylated) or conjugate an oligonucleotide. See Tech Tip #30: "Modify and label oligonucleotide 5′ phosphate groups"

Find these and other helpful tech tips at **thermofisher.com/protein-nucleic-interactions**

LightShift Chemiluminescent RNA EMSA Kit

Fast, nonradioactive detection of RNA–protein interactions

The Thermo Scientific™ LightShift™ Chemiluminescent RNA EMSA Kit provides a nonradioactive solution for studying RNA–protein interactions using an EMSA. An RNA EMSA is an *in vitro* technique that detects protein–RNA interactions through changes in gel electrophoresis migration patterns. A labeled RNA probe is incubated with a protein sample to initiate binding. Once a complex is formed, the sample is separated via nondenaturing 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.

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
- **Nonradioactive**—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 3´ End Biotinylation Kit (Cat. No. 20160). End-labeled RNA probes help 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 the cellular iron status. Under iron-starved conditions, IRP remains bound to the IRE RNA, suppressing translation of the iron-storage proteins, ferritin, and transferrin; 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. Incubating the positive control reaction with a 200-fold molar excess of unlabeled IRE RNA reduced the band-shift signal by 70%, indicating specificity; however, incubating the control reaction with a similar fold excess of an unrelated RNA did not significantly reduce the band shift (Figure 14). These results demonstrate the robustness, sensitivity and specificity of the IRE/IRP positive control.

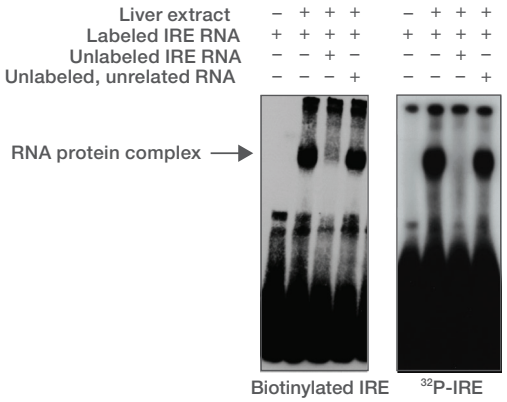


Figure 15. The IRE/IRP RNA EMSA positive control is sensitive and specific. Biotinylated IRE: For the binding buffer reaction, 5 nM (5.1 fmol) of biotinylated IRE RNA was incubated with 4 µg of cytosolic liver extract (containing IRP) in 1X REMSA binding buffer, 5% glycerol, and 2 µg of tRNA for 30 min 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.

In addition to avoiding radiolabeled nucleotides, a biotinylated RNA probe provides sensitivity comparable to radioactivity with faster detection. The sensitivity of a biotinylated RNA probe was compared to that of a radiolabeled probe by performing RNA EMSAs with different probe concentrations. For both biotinylated and radiolabeled probes, band shifts were robust and specific (Figure 15); 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 15).

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 UTPs. For the RNA polymerase binding reaction, the RNA template was end-labeled using the Pierce RNA 3´ End Biotinylation Kit (Cat. No. 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 16) 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.

The LightShift Chemiluminescent RNA EMSA Kit and radiolabeled EMSA performed comparably. 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 noncoding RNA–protein interactions, various RNA lengths and labeling methods, and purified protein as well as cell lysates.

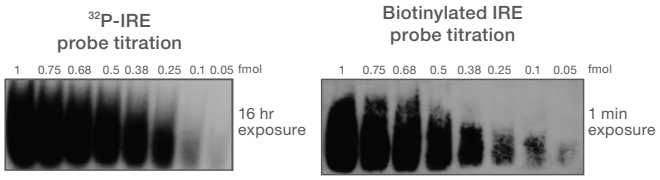


Figure 16. Chemiluminescent detection has comparable sensitivity to radioactive detection. The biotinylated IRE RNA probe was diluted to 50 amol. To generate ^{32}P-IRE, IRE (200 pmol) was labeled with γ-^{32}P ATP using T4 polynucleotide kinase, and then diluted to the same concentration as the biotinylated RNA.

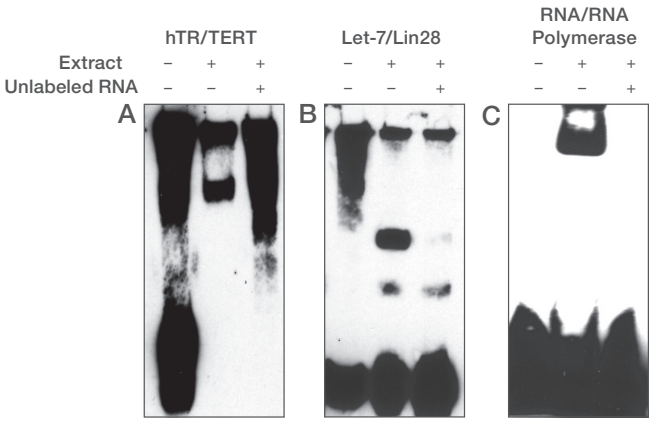


Figure 17. Chemiluminescent RNA EMSA accommodates different RNA and protein sources. Run-off transcription: Plasmid constructs were generated, and run-off transcription was performed using biotin-11-UTP or unlabeled UTP for hTR, and Let-7. **3´ end labeling:** RNA for the RNA polymerase reaction was 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.

Tech tip

Following exposure of a northern or Southern blot, researchers often wish to strip off the first probe and detecting reagents so that they may re-probe for the same or different target. Several commonly used conditions are described for stripping probe while maintaining the nucleic acid target on the membrane. See Tech Tip #28: "Strip northern and Southern blots"

Find these and other helpful tech tips at
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Pierce RNA 3′ End Desthiobiotinylation Kit

Easy and efficient RNA labeling and gentle elution from streptavidin

The Thermo Scientific™ Pierce™ RNA 3′ End Desthiobiotinylation Kit contains reagents for easy and efficient desthiobiotin labeling of RNA for use as probes or as antibody alternatives for enrichment of RNA binding proteins.

Highlights:

- **Nonradioactive**—a single biotin label has detection sensitivity similar to that of radioactivity
- **Easy to use**—RNA ligase and optimized reaction buffer are included
- **Economical**—only a fraction of the cost purchasing synthetic biotinylated RNA probes
- **End-labeled**—results in minimal disturbance of RNA secondary structure
- **Flexible**—desthiobiotin label may be used for detection or as an affinity handle for streptavidin resin

The Pierce RNA 3′ End Desthiobiotinylation Kit is optimized for labeling the 3′ end of single-stranded RNA using T4 RNA ligase. Once labeled, the RNA can be used as a probe or target for gel-shift EMSA reactions, northern blots and protein–RNA interaction experiments. The desthiobiotinylated RNA also can be used to enrich for RNA binding proteins (RBP) using streptavidin affinity resin, because the desthiobiotin tag binds to streptavidin in a manner that allows gentle elution of the ribonucleoprotein complex. The kit contains desthiobiotinylated cytidine bisphosphate, T4 RNA ligase and buffer, unlabeled RNA oligonucleotide for use as a positive control, a biotinylated RNA probe standard, RNase inhibitor, glycogen, and ligation-enhancing reagents for labeling. In addition, this labeling kit is also included as a component of the Thermo Scientific™ Pierce™ Magnetic RNA-Protein Pull-Down Kit.

The RNA labeling kit uses T4 RNA ligase to attach a single desthiobiotinylated cytidine bisphosphate to the 3′ end of single-stranded RNA. Each labeling reaction was designed for 50 pmol of RNA; however, reactions may be scaled (1 pmol to 1 nmol have been tested), if necessary. The reaction uses a 20-fold excess of desthiobiotinylated nucleotide and requires incubation times from 30 minutes at 37°C (for less complex RNA) to overnight at 4–16°C (for longer or more complex RNA). Optimization of the labeling efficiency for complex RNA structures is achieved by altering the RNA:nucleotide ratio, increasing the incubation time, or by adding DMSO to relax the RNA structure. After organic extraction and precipitation with ethanol, the desthiobiotin-labeled RNA is ready for use in downstream applications.

Applications:

- Northern blotting
- EMSA
- Enrichment of RNA binding proteins (RBP)

Table 5. Ligation efficiency of RNA desthiobiotinylation. RNA was heated to 90°C and held at that temperature for 5 min and then quickly chilled before ligation. Each reaction contained 50 pmol of RNA, and ligation reactions were performed according to the product instructions. Relative ligation efficiencies were determined by dot blot using densitometry and compared to a synthetic biotinylated RNA control (100%). Each ligation was performed in duplicate, and spotted in triplicate.

RNA	Length (nt)	Efficiency (%)
Control RNA	42	92
Poly(A)25	25	56
Androgen receptor	51	75
U1 RNA	46	70
Tat exon	57	90
Let-7 loop	40	50

Pierce Magnetic RNA-Protein Pull-Down Kit

Robust, easy to use kit to capture protein–RNA interactions

The Pierce Magnetic RNA-Protein Pull-Down Kit provides researchers with a streamlined, robust method to enrich protein–RNA interactions using end-labeled RNA as the bait.

Highlights:

- **Direct**—capture ribonucleoprotein complexes directly with end-labeled RNA; does not use or require antibodies for pull-down
- **Easy to use**—no spin cups or centrifugation necessary for the enrichment of RBP; procedure streamlined for minimal hands-on time (typically less than 3 hours) after RNA labeling reaction
- **Flexible**—use *in vitro*–transcribed RNA or synthetic RNA for labeling of various lengths and complexity; proteins successfully enriched using endogenous, over-expressed, and *in vitro*–translated lysates
- **Specific**—low bead background; unrelated RNA or mutated RNA does not significantly enrich specified RBPs
- **Economical**—less expensive than purchasing commercially synthesized end-labeled RNA, magnetic beads, and reagents separately
- **Complete**—contains both labeling and enrichment modules with buffers necessary for assay; positive control RNA, negative control RNA, and RBP antibody included

The Pierce Magnetic RNA-Protein Pull-Down Kit provides reagents to efficiently enrich RBPs using RNA, end-labeled with desthiobiotin and streptavidin magnetic beads. The complete kit contains the Pierce RNA 3´ End Desthiobiotinylation Kit, positive and negative RNA controls, nucleic acid–compatible streptavidin magnetic beads, and buffers for RBP enrichment and elution, sufficient for 20 RNA labeling reactions and 20 protein–RNA pull-down assays. This direct enrichment of the protein–RNA interaction provides an alternative to antibody capture of protein–RNA complexes or moieties incorporated into the nucleic acid. An added advantage of the kit is that it includes verified controls for both the labeling and pull-down assay. The kit is amenable to several downstream applications, including western blotting and mass spectrometry (MS).

Utilizing labeled RNA as a bait for RNA-binding protein enrichment is advantageous over antibody enrichment in that it captures the interaction directly. Included in this kit is the Pierce RNA 3´ End Desthiobiotinylation Kit, which uses T4 RNA ligase to attach a single cytidine bisphosphate nucleotide to the 3´ ends of single-stranded RNA. Desthiobiotin may be used for detection or as an elution-compatible affinity handle. The length of the spacer between the nucleotide and desthiobiotin has been optimized for efficient attachment to the beads without compromising the accessibility of the RNA to protein.

The procedure for enrichment of RNA binding proteins has been optimized for ease of use. The labeled RNA is first captured to the beads to orient the RNA for protein binding. RNA-bound beads are then equilibrated in Protein-RNA Binding Buffer before protein lysate is added. After washing, sample can be eluted using either nondenaturing Biotin Elution Buffer or SDS-PAGE loading buffer. Eluted samples are ready for a variety of downstream applications, including western blotting and mass spectrometry.

The control system for the pull-down assay utilizes 3´-untranslated region androgen receptor (AR) RNA, poly(A)25 RNA, and mammalian cell lysate. The proximal 3´-untranslated region (UTR) of androgen receptor RNA contains UC-rich regions for HuR and Poly(C) Binding Proteins (CP1 and 2). These RNA-binding proteins regulate mRNA stability (HuR), and mRNA turnover and translation (CP1 and 2). The negative control RNA, poly(A)25 RNA, does not contain HuR- or poly(C) BP-binding sites.

Applications

- Mutational analysis
- Structure function analysis
- Identification of RNA–protein interactions
- MS analysis of unknown RNA–protein binding pairs or complexes

Figure 18. Let-7 loop RNA specifically enriches Lin28 in NCCIT extract. Desthiobiotinylated let-7 miRNA (50 pmol) was used to enrich Lin28 using the Pierce Magnetic RNA-Protein Pull-Down Kit (Cat. No. 20164), followed by western blotting. Samples were normalized by volume. L = lysate; FT = flow-through; E = eluate. Unrelated: negative control poly(A)25 RNA.

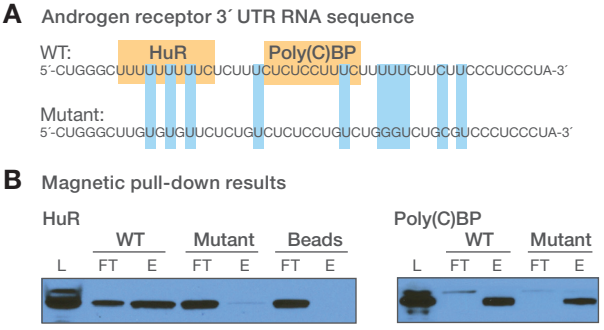

Figure 19. Example RNA–protein pull-down experiment. The effect of mutation on the ability of androgen receptor 3′-UTR RNA to bind HuR and Poly(C)BP. Wild type (WT) and mutant AR 3′-UTR RNA (50 pmol) were labeled and used for the pull-down assay according to the kit procedure. Samples were normalized by volume. L = lysate; FT = flow-through; E = eluate. PCBP1 antibody (1:1,000).

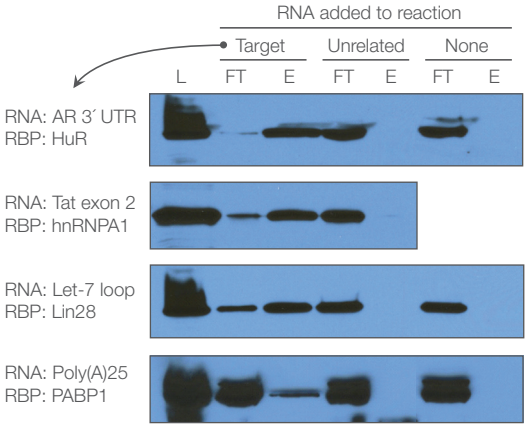

Figure 20. End-labeled RNA enriches specific target binding proteins. The RBPs of the AR 3′ UTR control system (top panel) and three experimental systems were enriched according to kit procedure. L = lysate; FT = flow-through; E = eluate. Incubation of A431 lysate with labeled AR UTR RNA (target) enriches HuR RBP, while incubation with negative control poly(A) RNA (unrelated) or beads only (none) does not (compare elution lanes). The same pattern results with the experimental systems, confirming the proper function of the kit. Samples were normalized by volume, and bands were detected using Thermo Scientific™ SuperSignal™ West Pico Substrate (Cat No. 34080) by a 2 min exposure to film. Target RNA sequences were as follows:

Androgen receptor 3′ UTR (kit control system):
5′-CUGGGCUUUUUUUUUCUCUUUCUCUCCUUUCUUUUUCUUCUUCCCUCCCUA-3′

Tat exon 2:
5′-UUACUCAACAGAGGAGAGCAAGAAAUGGAGCCAGUAGAUCCUAGACUAGAGCCCUGG-3′

Let-7 loop:
5′-CAGUUUGAGGGUCUAUGAUACCACCGGUACAAGAUAACUG-3′

Poly(A)25:
5′-AAAAAAAAAAAAAAAAAAAAAAAAA-3′

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Ordering information

Product	Quantity	Cat. No.
Sample lysis and protein extraction		
NE-PER Nuclear Protein Extraction Kit	Kit	78833
M-PER Mammalian Protein Extraction Reagent	500 mL	78501
T-PER Tissue Protein Extraction Reagent	500 mL	78510
Inhibitor cocktails and tablets		
Halt Protease Inhibitor Cocktail (100X)	1 mL	87786
Halt Protease Inhibitor Cocktail (100X), EDTA-free	1 mL	87785
Pierce Protease Inhibitor Mini Tablets	30 tablets	A32953
Pierce Protease Inhibitor Tablets	20 tablets	A32963
Pierce Protease Inhibitor Mini Tablets, EDTA-free	30 tablets	A32955
Pierce Protease Inhibitor Tablets, EDTA-free	20 tablets	A32965
Halt Phosphatase Inhibitor Cocktail (100X)	1 mL	78420
Pierce Phosphatase Inhibitor Mini Tablets	20 tablets	A32957
Halt Protease and Phosphatase Inhibitor Cocktail (100X)	1 mL	78440
Halt Protease and Phosphatase Inhibitor Cocktail (100X), EDTA-free	1 mL	78441
Pierce Protease and Phosphatase Inhibitor Mini Tablets	30 tablets	A32959
Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-free	30 tablets	A32961
Protein–DNA interaction products		
Biotin 3´ End DNA Labeling Kit	Kit	89818
LightShift Chemiluminescent DNA EMSA Kit	Kit	20148
MAGnify Chromatin Immunoprecipitation System	24 reactions	492024
Pierce Agarose ChIP Kit	30 reactions	26156
Pierce Magnetic ChIP Kit	30 reactions	26157
Pierce ChIP-Grade Protein A/G Magnetic Beads	5 mL	26162
Pierce Chromatin Prep Module	Kit	26158
Protein–RNA interaction products		
Pierce RNA 3´ End Biotinylation Kit	Kit	20160
Pierce RNA 3´ End Desthiobiotinylation Kit	Kit	20163
LightShift Chemiluminescent RNA EMSA Kit	Kit	20158
Pierce Magnetic RNA-Protein Pull-Down Kit	Kit	20164
Pierce Streptavidin Magnetic Beads	5 mL	88817
Related products		
Chemiluminescent Nucleic Acid Detection Module	Kit	89880
DynaMag-2 Magnet	1 each	12321D
NF-κB p50 Transcription Factor Kit	2 plates/kit	89858
NF-κB p65 Transcription Factor Kit	2 plates/kit	89859
Nucleic Acid Detection Blocking Buffer	500 mL	89880A
tRNA, 10 mg/mL	100 mL	20159
Biodyne B Nylon Membranes, 0.45 µm, 8 cm x 12 cm	25 sheets	77016
Proteinase K (20 mg/mL)	0.25 mL	26160

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