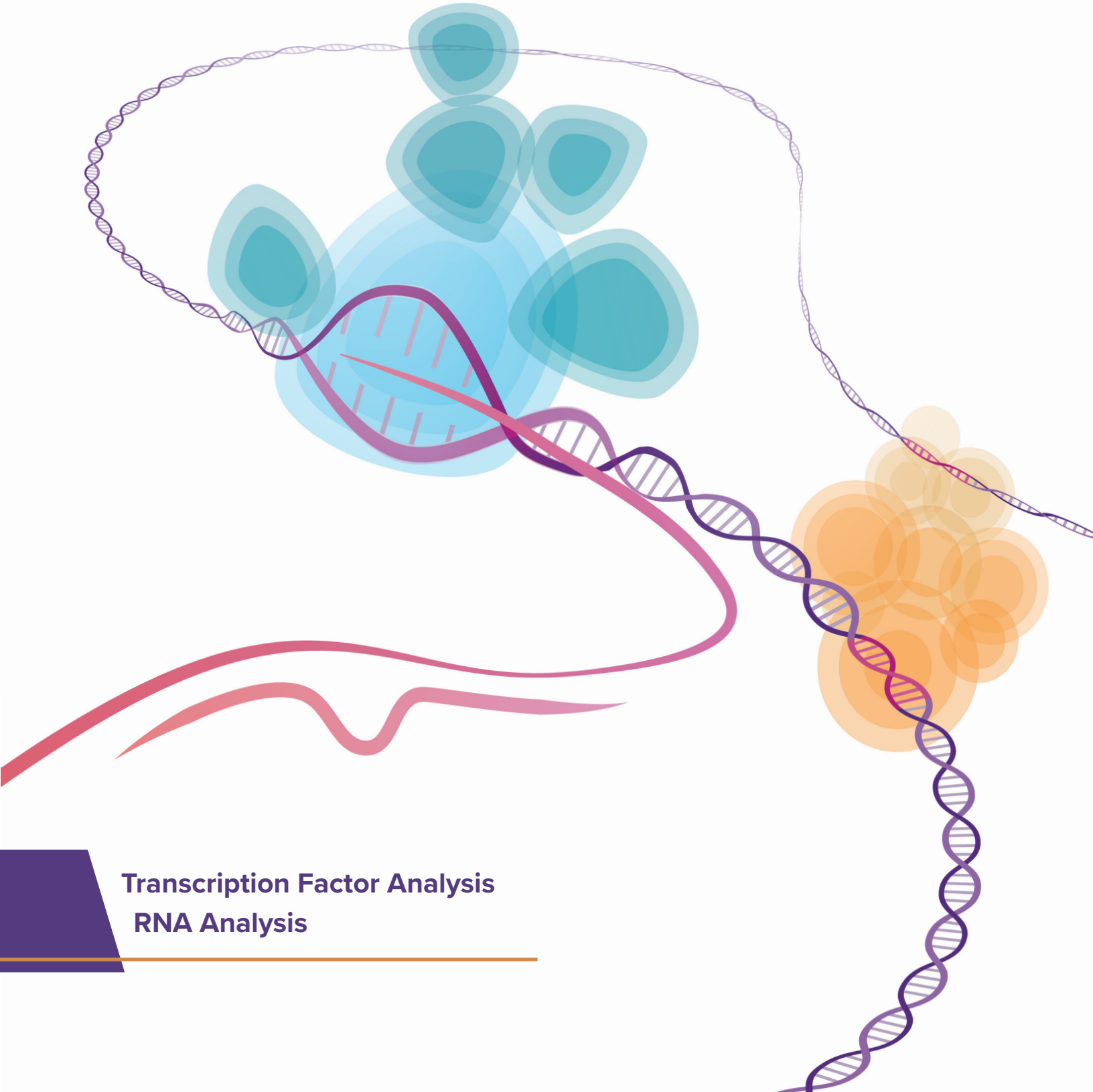
ACTIVE MOTIF®

Enabling Epigenetics Research

Gene Regulation

Products & Services



Transcription Factor Analysis

RNA Analysis

Gene Regulation overview

The Dynamic Process of Gene Regulation

Gene regulation is a highly dynamic process that involves not only transcription factor (TF) and effector protein interactions with DNA at promoters and enhancer regulatory regions, but also chromatin remodeling events driven by epigenetic mechanisms. These mechanisms include histone modifications, DNA methylation, and non-coding RNAs that influence accessibility of transcriptional machinery to underlying DNA.

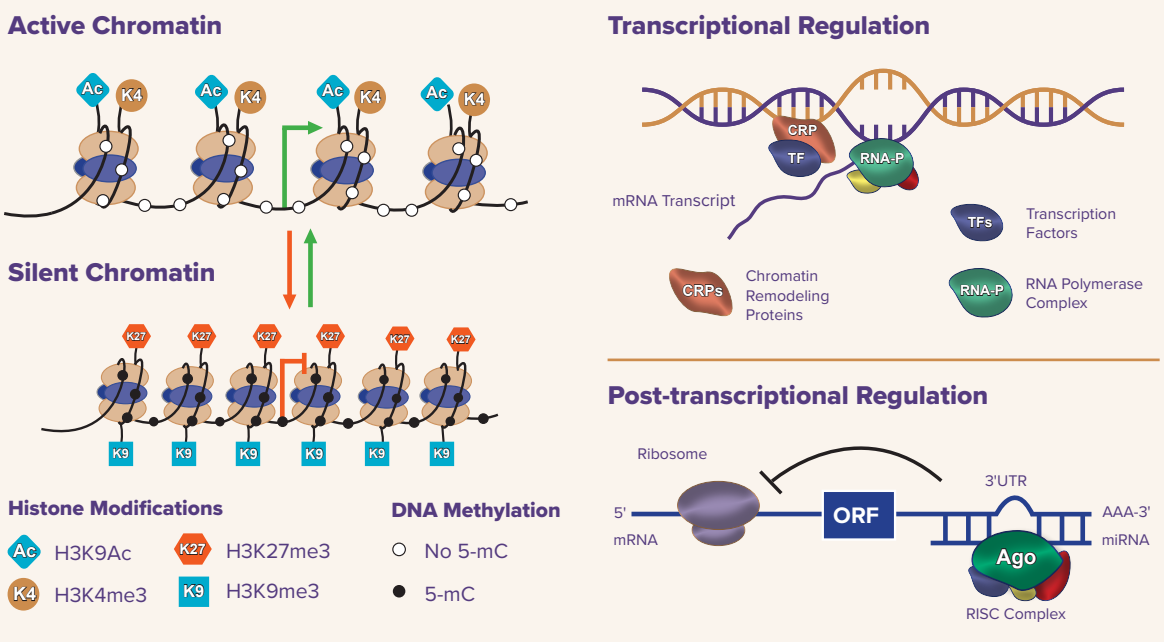
All of these mechanisms work in concert to regulate gene expression. Furthermore, once genes are transcribed, microRNAs and other mechanisms play critical roles in modulating gene expression at the post-transcriptional level, adding yet another layer of complexity to the process.

Tools to Study Gene Regulation

Active Motif is the industry leader in providing innovative tools to enable epigenetics and gene regulation research. Our superior products, services, and support serve our life science, clinical, pharmaceutical, and drug discovery partners. Our selection of products to study gene regulation includes TransAM® transcription factor binding assays and RNA analysis kits and services.

Whether you are already an expert in gene regulation or are just looking to get started, our comprehensive portfolio of products offers End-to-End solutions to meet the specific needs of your research.

Epigenetic Modifications



To learn more, visit us at **activemotif.com**

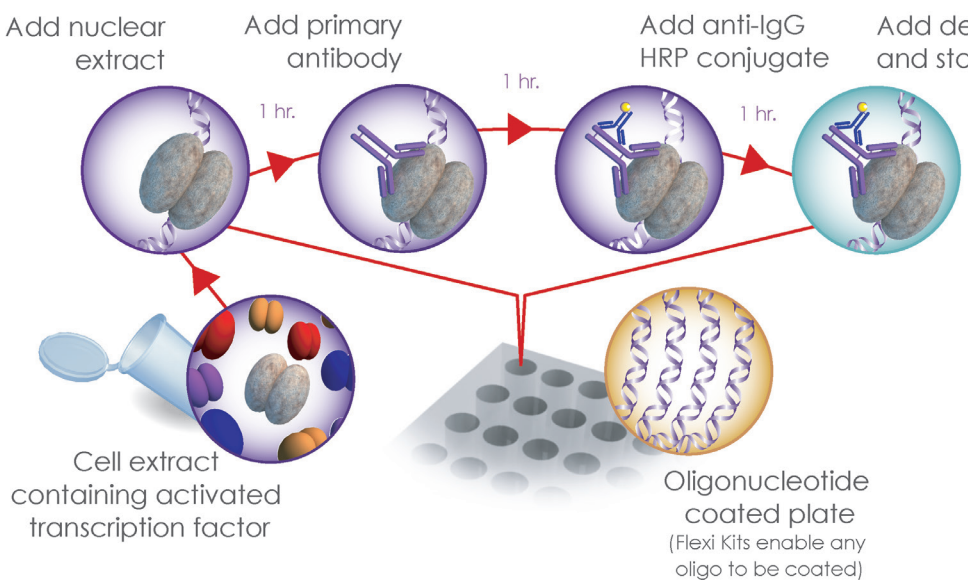
Transcription Factor Analysis

TransAM® Transcription Factor Activation Assays

TransAM transcription factor assays are fast, user-friendly, and highly sensitive non-radioactive ELISA-based assays that facilitate the study of transcription factor-DNA binding activity in mammalian tissue and cell extracts. TransAM assays are up to 100-fold more sensitive than gel shift assays and also yield more quantitative results.

Advantages of TransAM Transcription Factor Assays

- Up to 100-fold more sensitive than gel shifts
- Results in fewer than 5 hours
- Non-radioactive, colorimetric readout
- No cloning or transfection required
- 96-stripwell format for scalability



Flow chart of the TransAM process.

Activated transcription factor in cell extract binds to the consensus-binding site on the oligo immobilized in the well. Incubation with the supplied primary and secondary antibodies specifically quantifies the amount of activated transcription factor.

Our TransAM Assay Kits have been cited in more than 1,000 publications

Select from the list of available transcription factors below and upgrade your transcription factor activation assays.

Available Transcription Factor Assays

AP-1	IRF-3
ATF-2	MAPK Family
c-Myc	NFATc1
C/EPB	NFkB
CREB & pCREB	Nrf2
ER	p53
FKHR (FOXO1)	PPARy
GR	Sp1 & Sb1/Sp3
HIF-1	T-bet
HNF	

Active Motif offers many different TransAM assays and formats, along with our Nuclear Extract Kit (cat. nos. 40010 & 40410) for sample preparation and recombinant transcription factors for standard curve generation.

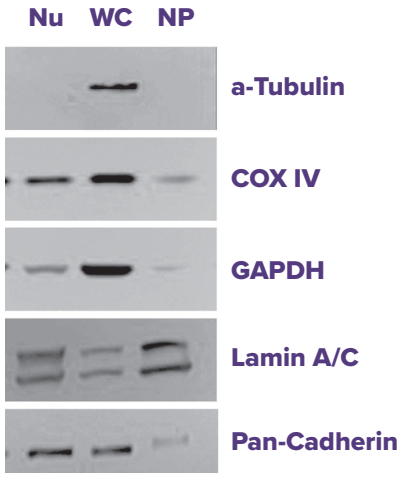
For a complete up-to-date list of available TransAM products and detailed information, visit us online at **activemotif.com/transam**

Nuclear Extract Kit

Active Motif's Nuclear Extract Kit offers a simple method for the preparation of highly pure nuclear, cytoplasmic, and whole-cell extracts from mammalian cells and tissues.

Use of the Nuclear Extract Kit eliminates the need to optimize reagents and ensures consistent sample preparation. The nuclear extracts generated with this product are of high yield, concentration, and purity, providing you with the most optimal purified protein fractions for analysis by various methods including Active Motif's TransAM® kits, electrophoretic mobility shift assays (EMSAs), and western blots.

Use the Nuclear Extract Kit to improve assay results



Easy and efficient sub-cellular fractionation with the Nuclear Extract Kit

Efficiency of cellular fractionation was determined by collecting 20 µg of nuclear (Nu), and whole-cell (WC) extracts, along with nuclear pellet (NP) using the Nuclear Extract Kit and performing Western blot using antibodies against various specific proteins.

Key Highlights

- **Fast and Easy** - Streamlined 2-hour protocol that requires only standard laboratory equipment
- **Scalable** - 100 and 400 reaction kit formats available
- **Flexible** - Prepare nuclear, cytoplasmic, or whole-cell extracts starting from cells or tissues
- **Reliable** - Quality-controlled reagents to ensure reproducibility

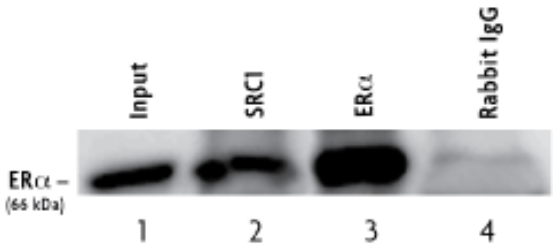
Universal Magnetic Co-IP Kit

The Universal Magnetic Co-IP Kit enables greater flexibility for analysis by Co-IP by providing reagents for isolation of native protein complexes from either nuclear or whole-cell lysates prepared from cells or tissue.

The co-immunoprecipitation procedure utilizes protein G-coated magnetic beads for more rapid and efficient IP and wash steps. The beads also greatly reduce background and minimize sample loss for better recovery and improved results from your Co-IP experiments.

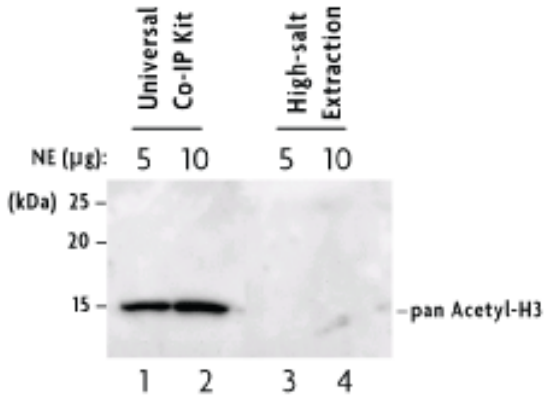
Improve your Co-IP assays with the Universal Magnetic Co-IP Kit

- **Flexible** - Perform Co-IP of nuclear or cytoplasmic protein complexes
- **Gentle** - Preserves protein interactions & modifications
- **Fast** - Magnetic beads streamline procedure & reduce background
- **Complete** - Includes reagents for both extraction & IP procedure



By using magnetic beads, it's possible to use low stringency buffers while maintaining reduced background.

The Universal Magnetic Co-IP Kit was used to make nuclear extract from MCF-7 cells that had been induced 1 hour with 10 nM Estradiol. IP was performed on 300 µg samples using 2 µg of SRC-1 pAb, ERα pAb and rabbit IgG (as a negative control). Western blot was then performed using the ERα pAb on 10 µg Input Extract, SRC-1 IP, ERα IP and the rabbit IgG IP.



Proprietary Enzymatic Shearing Cocktail releases undissociated protein complexes from the DNA so that even histone complexes can be studied.

HeLa nuclear extracts were made using the Universal Magnetic Co-IP Kit or a traditional high-salt extraction protocol, supplemented with 1 µM trichostatin A, a deacetylase inhibitor. Five and ten µg samples of each extract were used in Western Blot with a Histone H3 acetyl pAb. Protein was detected only in samples made using the kit's nuclear extraction procedure, as it was designed to release histone and other protein complexes from DNA while preserving modifications.

RNA Analysis

RNA Analytical Services

Historically the function of RNA in the cell was thought to be primarily a messenger that translated information from DNA into protein. We now know that RNA is involved in a diverse array of biological processes including direct catalysis and a multitude of regulatory mechanisms.

Active Motif offers two main gene expression services

Our comprehensive End-to-End RNA analysis services makes it easy and efficient to incorporate genome-wide transcriptomic studies into your research.

We offer two main services to investigate gene expression on a genome-wide scale; RNA-Seq for identification and quantitation of RNA transcripts, and RNA Pol II ChIP-Seq for quantitation of transcription rates to enable rapid profiling of changes in gene expression associated with transcription factor (TF) and histone modification occupancy.

RNA Pol II ChIP-Seq Services

Analysis of RNA Pol II occupancy as a proxy measurement of transcription rates offers the advantage of enabling you to:

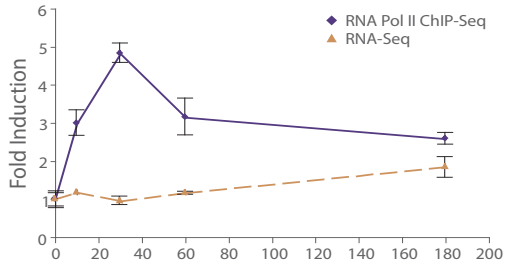
- Measure transcription without the influence of RNA half-life
- Identify genes poised for transcriptional activation
- Generate gene expression data from cells used for ChIP-Seq
- Measure changes at early time points post-treatment
- The impact of miRNA inhibitors or stimuli

RNA-Seq Services

Simply submit RNA, cells, or tissue samples. Order RNA-Seq alone or combine with ChIP-Seq data to uncover contextual information about:

- Differential gene expression
- Changes in gene structure or splicing patterns
- Effects of TF binding on gene expression
- Effect of sequence variants on 3'UTR or miRNA function
- The impact of miRNA inhibitors or stimuli

RNA Pol II Changes Are Detected Earlier Than mRNA Changes



Gene expression profiles vary depending on the analysis method.

Data for IGF1R expression was extracted from RNA–Seq and RNA Pol II ChIP-Seq data sets. Cell treatment resulted in induced gene expression that was measured at various time points. The data show that transcription, as measured by RNA Pol II ChIP-Seq, is induced immediately, while mRNA levels only accumulate over time.

Key Features

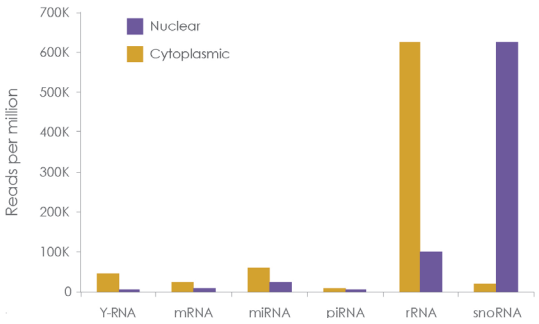
- PolyA enrichment
- Directional library preparation
- Paired-end sequencing on Illumina® sequencing platform
- QC performed using Bioanalyzer
- Data analysis pipelines include differential analysis and GSEA

RNA Subcellular Isolation Kit

Active Motif's RNA Subcellular Isolation Kit is designed to efficiently isolate separate nuclear and cytoplasmic RNA fractions for downstream analysis. This method can be used to isolate RNA molecules larger than 75 nucleotides, including long non-coding RNAs (lncRNAs), ribosomal RNAs, messenger RNAs, and small nucleolar RNAs (snoRNAs) from cells or tissue without cross-contamination or the use of phenolic compounds.

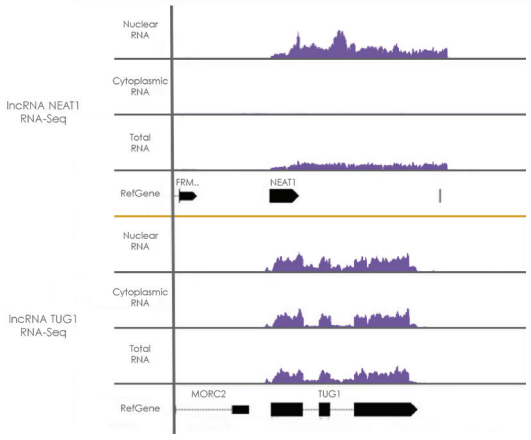
Advantages of the RNA subcellular isolation kit

- **Works with cells and tissues**
- **Method avoids the use of phenol**
- **Isolates RNA greater than 75 nt, including lncRNAs, snoRNAs, rRNAs, mRNAs, and more**
- **Enhanced detection of low abundance transcripts**
- **Reduced background from other intronic or mature RNAs**
- **Purified RNA is validated for use in RT-qPCR and RNA-Seq**



Use the RNA Subcellular Isolation Kit to easily identify small RNA distributions.

Cytoplasmic and nuclear RNA were isolated from HeLa cells. RNA was processed and size selected to remove large RNA. 75 bp single read sequencing was performed and the data was mapped to miRBase. A frequency of the small RNA (sRNA) distribution for each fraction shows the specificity of subcellular isolation.

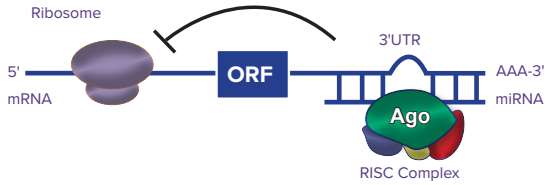


Focus your RNA-Seq on the relevant sub-cellular fractions

Nuclear, cytoplasmic and total RNA were isolated from HeLa cells. RNA was subjected to ribosomal RNA depletion during NGS library preparation. Samples were then sequenced using the Illumina® HiSeq and 100 bp paired end reads with 50 M reads per sample. NEAT1 is a lncRNA that is primarily located in the nucleus, while TUG1 is a lncRNA known to have both nuclear and cytoplasmic localizations.

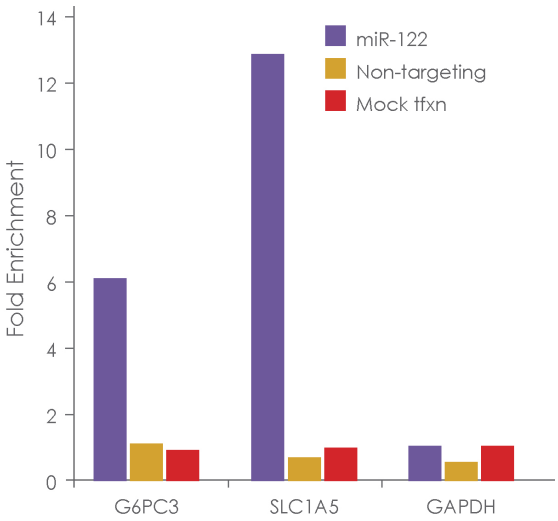
miRNA Target IP Kit

Active Motif's miRNA Target IP Kit was designed to capture and identify the physical interactions of miRNAs with endogenous mRNA transcripts for validation of the binding targets of specific miRNAs. The targeting of a microRNA (miRNA) to a specific mRNA is mediated through the formation of an RNA Induced Silencing Complex (RISC), containing a combination of various RNA-binding proteins along with the Argonaute (Ago) protein and miRNA. The Active Motif miRNA Target IP Kit utilizes a pan-Ago antibody that recognizes Ago1, Ago2 and Ago3 proteins for immunoprecipitation (IP) of miRNA/mRNA interactions as part of the RISC complex.



The miRNA within a RISC complex enables precise silencing of specific mRNA transcripts.

The key components in a RISC complex are an Ago protein and a miRNA. The Ago protein binds the miRNA, positioning it in a conformation that enables the RISC to base-pair in a Watson-Crick manner with a mRNA transcript. This leads to either inhibition of translation (shown) or increased degradation of the targeted transcript.



Identify miRNA target genes.

The miRNA Target IP Kit was used on HT1080 cells transfected with either a miR-122 mimic, a non- targeting miRNA control, or a mock plasmid control. Following IP using the Ago1/2/3 antibody or Negative Control IgG included in the kit, qRT-PCR was performed using primers for G6PC3 and SLC1A5, which are known targets of miR-122, and GAPDH, a common housekeeping gene that is not known to be targeted by miR-122.

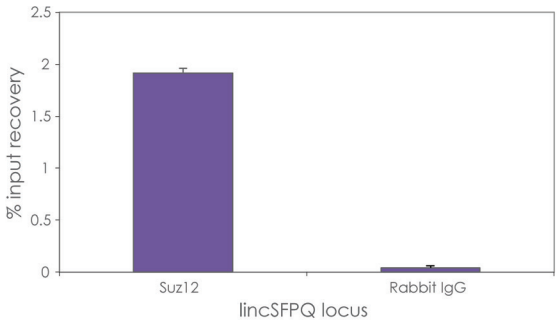
RNA ChIP-IT® Kit

Isolate and study RNA-protein complexes specifically from chromatin

To facilitate the characterization of the role of RNA in genome regulation and organization, Active Motif has applied the efficient magnetic bead-based protocol used to develop our ChIP-IT Express Kits to develop the first of its kind kit for RNA ChIP. The RNA ChIP-IT Kit was designed to specifically enable investigation of RNA-protein interactions in a chromatin context and has been optimized for RNA.

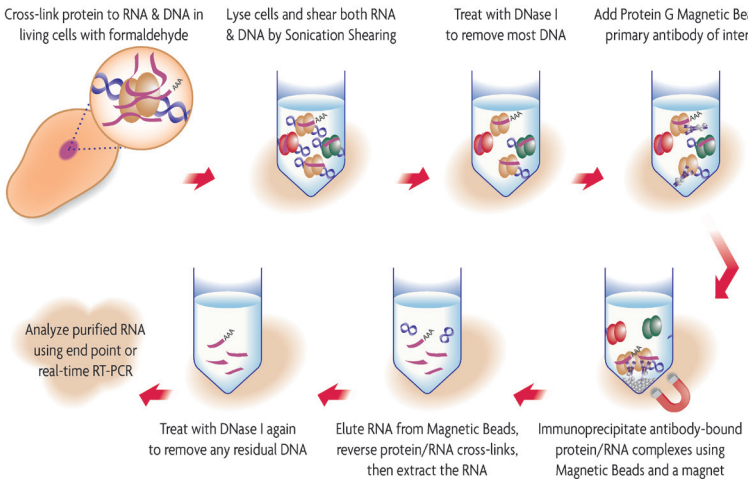
Key Features

- **Specifically tailored to study chromatin-associated RNA**
- **Designed to remove DNA while maintaining RNA integrity**
- **Step-by-step protocols for fixation of chromatin, sonication and immunoprecipitation, all optimized for RNA preservation**
- **Separate control kit available with control antibody and primers**



Study chromatin-associated RNAs.

The RNA ChIP-IT Kit was used on 10 µg samples of DNase I-treated HeLa chromatin with 10 µl of Suz12 antibody (Cat. No. 39357) and 2 µg of normal rabbit IgG. The RNA-IP was performed overnight at 4°C. Real-time RT-PCR was performed using primers for the lincRNA SFPQ locus.



Flow chart of the RNA ChIP-IT method

Ordering Information

Product	Cat. No.
Nuclear Extract Kit	40010
Universal Magnetic Co-IP Kit	54002
Gelshift Chemiluminescent EMSA kit	37341
Nuclear Complex Co-IP Kit	54001
RNA Subcellular Isolation Kit	25501
miRNA Target IP Kit	25500
RNA ChIP-IT® Kit	53024
mTRAP™ Total	23012
mTRAP™ Midi	23024
mTRAP™ Maxi	23006
p53 antibody (mAb)	39553
YY1 antibody (pAb)	61779
AbFlex® MAZ antibody (rAb)	91205
AbFlex® MITF antibody (rAb)	91201
HIF-1 alpha antibody (pAb)	39665
N6-Methyladenosine (m6A) antibody (mAb)	61755
AbFlex® N6-Methyladenosine (m6A) antibody (rAb)	91261
CTCF antibody (pAb)	61311
DNMT1 antibody (mAb)	61467
p53 antibody (mAb)	39553
AbFlex® RNA Pol II antibody (rAb)	91151
RNA pol II CTD phospho Ser2 antibody (mAb)	61083
Recombinant NFkB1 p50 (1-434) protein	81032
Recombinant NFkB1 p105 protein	81143
Recombinant NFkB3 (RELA / p65) protein	81086
Recombinant p53 (TP53) protein	81091
Recombinant YY1 protein	81119
Recombinant MAX protein	81017
Recombinant c-Myc / MAX complex	81087
Recombinant IKKβ protien	81066
Recombinant ALKBH5 protein	31589
Recombinant FTO protein	31572
Recombinant METTL3/METTL14 complex	31570
Recombinant RNA Pol II - CTD protein	81036

Take advantage of Active Motif Epigenetics Resources!

Epigenetics 101: Complete Guide to Understanding Epigenetics
Take a deep dive into epigenetics in our comprehensive learning center.

Epigenetics Blog
Follow our posts about the latest trends in epigenetics research.

eBooks
Stay up to date with the latest eBooks from our epigenetics experts.

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Watch the latest tips and techniques from our epigenetics experts.

Videos
Browse our video collection and become an epigenetics expert.

Chromatin Clubs & Epigenetics Events
Keep connected to the epigenetics research community with chromatin clubs, online seminars, virtual conferences, and more epigenetics events.

Check out the chromatin club events that we have scheduled or contact us to request support for a chromatin club you are planning.

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