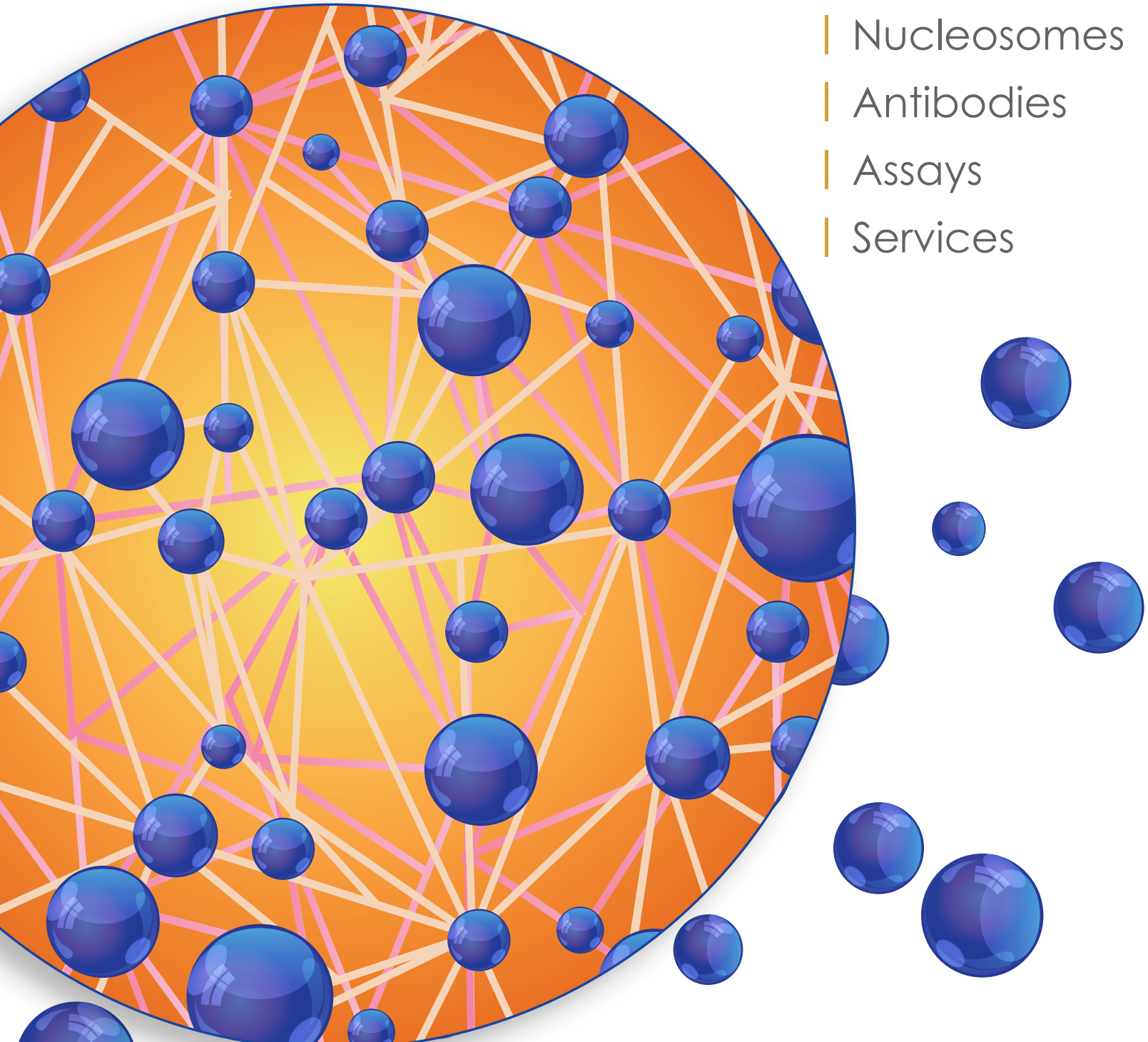
ACTIVE MOTIF®

TOOLS FOR EPIGENETIC DRUG DISCOVERY

- Proteins
- Nucleosomes
- Antibodies
- Assays
- Services



DRUG DISCOVERY TOOLBOX

assays, reagents & services to accelerate your research

Epigenetic drug discovery & development programs have attracted much attention and investment in recent years. This is driven by the potential to develop novel therapeutic strategies that can reverse transcriptional and epigenetic abnormalities.

Active Motif is the industry leader in providing innovative technologies for epigenetics and gene regulation research. This brochure features assays, reagents & services that can easily be integrated into your research strategy to advance and streamline the drug discovery workflow.


Assay-ready Enzymes


Histone Substrates


Recombinant Nucleosomes


Small Molecules


Target-relevant Antibodies


Histone Peptide Array


Multiplex Epigenetic Assays


Global Response Assays


Genome-wide Services


Pathway Analysis


miRNA Functional Assays


Screening Tools

For a complete list of available products, please visit us at www.activemotif.com.

Tools for Drug Discovery

proteins, substrates, antibodies & assays for epigenetics drug discovery

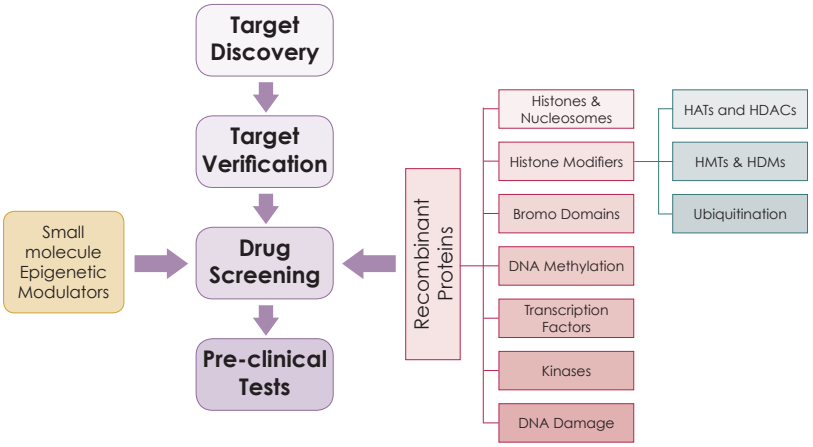
Epigenetic targets are the most promising class of druggable targets to emerge in a decade. These targets are not only relevant to oncology research, but they also have potential impact on metabolic, neurological, inflammatory and cardiovascular disorders. Active Motif offers everything from highly pure, validated antibodies and proteins to fully developed assays and services to help advance research in all phases of drug discovery.

> 700 VALIDATED ANTIBODIES

- Histone modifications
- Chromatin modifiers
- DNA methylation
- DNA damage
- Transcription regulation
- Stem cell
- Nuclear receptors
- Signal transduction

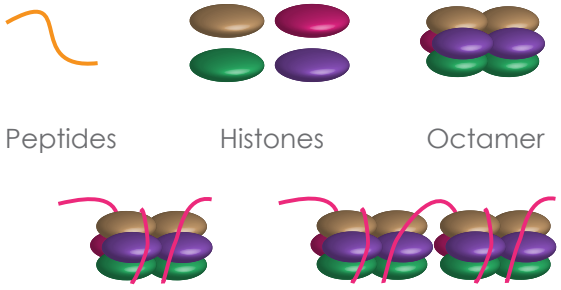
> 300 PURIFIED ASSAY-READY PROTEINS

- Bromodomains
- Chromatin modifiers
- Methyltransferases
- Demethylases
- Acetyltransferases
- Deacetylases
- Transcription factors
- Nuclear receptors



Active motif offers a large collection of high quality, robust epigenetic modifying enzymes and substrates that are ideally suited for use in drug discovery for the development of activity assays for drug screening applications.

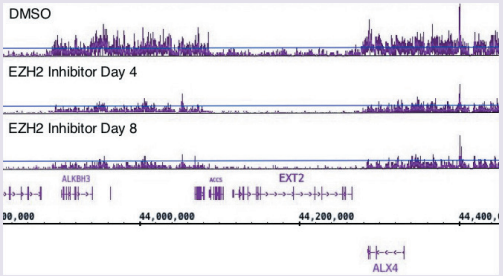
PHYSIOLOGICALLY-RELEVANT ASSAY SUBSTRATES



Peptides | Histones | Octamer

Mono nucleosome | (Di, Tri ...) Oligo Nucleosomes

IDENTIFY MECHANISM OF ACTION AT THE EPIGENETIC LEVEL



Active Motif offers a suite of Services specifically designed to enhance drug development programs, especially those focused on small molecule epigenetic inhibitors. In the early phases of drug discovery, our recombinant proteins and cell-based screening services will accelerate your program, while in the late phase our genomic assays, such as ChIP-Seq, RNA-Seq, MeDIP-Seq and Open Chromatin-Seq will lead to a greater understanding of your compound's mechanism of action.

For a complete list of available purified recombinant proteins, please visit us at www.activemotif.com/proteins.

Epigenetic Proteins

highly purified recombinant proteins for epigenetics research

One of the current challenges in the field of epigenetics research and drug discovery is developing robust enzymatic assays. The key to achieving a robust enzymatic assay is the ability to obtain high quality proteins.

Active Motif has developed a large selection of full-length high quality, robust epigenetic modifying enzymes for use in the development of activity assays for drug discovery. Our offerings include N-terminal tagged versions as well as several protein complexes, including PRC2 complex and MLL complex. Our proteins have been tested in a variety of highly sensitive enzyme activity assay technologies, including AlphaLISA, HTRF, fluorography and mass spectrometry.

> 360 PROTEINS AVAILABLE

- Full length proteins and catalytic domains
- Tagged proteins
- Protein complexes
- Custom and bulk production
- Activity by HTRF and AlphaLISA
- Purity by SDS-PAGE

READERS

- BRD1
- BRD4
- BRD9
- BAZ1B
- SMARCA4
- TRIM24

WRITERS

- PRMT
- p300
- EZH2
- DOT
- SET
- SMYD

ERASERS

- HDAC
- JMJ
- LSD1
- KDM5A/B
- UTX
- TET

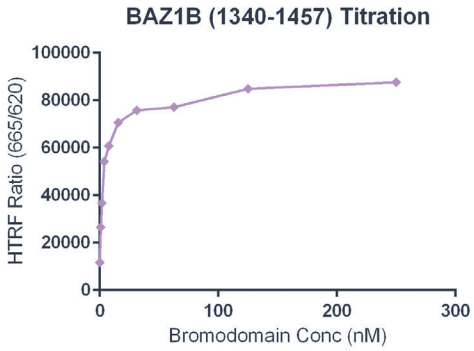


Figure 1: Recombinant BAZ1B (1340-1457) HTRF activity assay. 3.3 µM histone peptide H4K5/8/12/16(4Ac) was incubated with BAZ1B (1340-1457) protein in reaction buffer including 50 mM HEPES-NaOH pH 7.0, 0.1% BSA overnight at room temperature. Anti-FLAG antibody was used to detect reaction products.

Purification of Full-length Recombinant Proteins

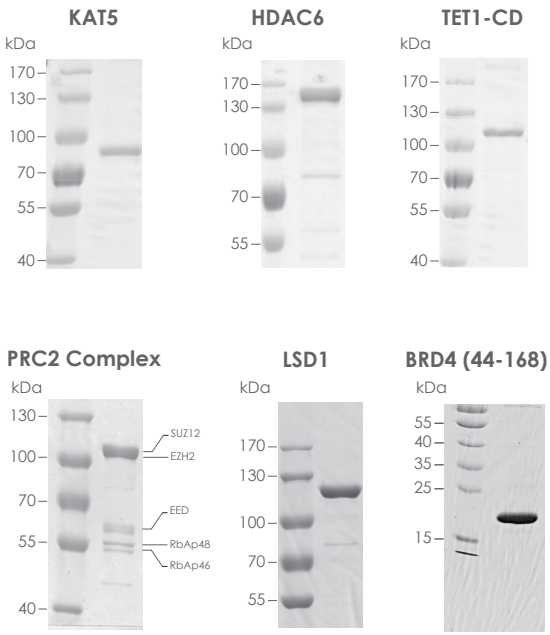


Figure 2: Full-length recombinant proteins and protein complexes expressed and purified. The Coomassie stains reveal that recovered proteins are full length with little or no detectable degradation and determined to have ≥ 98% purity.

IN-HOUSE MANUFACTURED AND QC'D

- Custom & bulk orders available
- Please contact us at www.activemotif.com/epiproteins

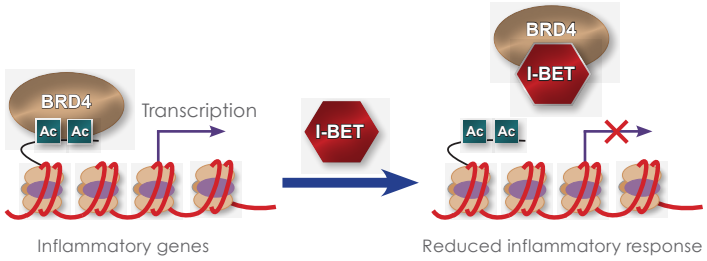
For a complete list of available purified recombinant proteins, please visit us at www.activemotif.com/proteins.

Bromodomain Proteins

the most comprehensive reader domain collection available for drug discovery

Bromodomain proteins play an integral role in the regulation of transcription and chromatin remodeling. Because bromodomain proteins have been shown to also regulate transcription of certain oncogenes, they are promising therapeutic targets for cancer treatment. To support research efforts, Active Motif offers a comprehensive collection of recombinant bromodomains for drug discovery research. Our recombinant proteins are manufactured to meet the highest standards of purity and activity required for conventional drug discovery assays, such as HTRF (Figure 1), mass spectrometry, AlphaLISA and Alphascreen. These assays are routinely incorporated as part of our quality control process to ensure that our recombinant proteins and enzymes demonstrate the nM range activities needed for use in identification of potent inhibitors.

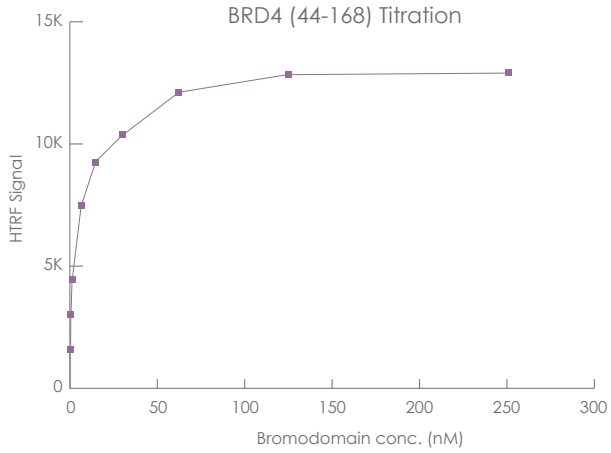
DESIGNED SPECIFICALLY FOR USE IN ASSAYS AND SCREENS



ACTIVE MOTIF BROMODOMAIN PROTEINS

- Over 40 reader domains available
- Designed for epigenetic drug discovery
- Tagged versions available
- Lot specific data, including:
  - activity by HTRF and AlphaLISA
  - purity by SDS-PAGE

Product	Catalog No.
BRD1 (556-688)	31438
BRD3 (24-144)	31379
BRD4 (44-168)	31380
BRD7 (129-236)	31381
BRD9 (130-259)	31382
BRDT (21-137)	31450
BRPF1 (627-746)	31375
CREBBP (1081-1187)	31373
SMARCA2 / BRM (1367-1511)	31449
TAF1 (1398-1524)	31403
TRIM24 (862-980)	31368



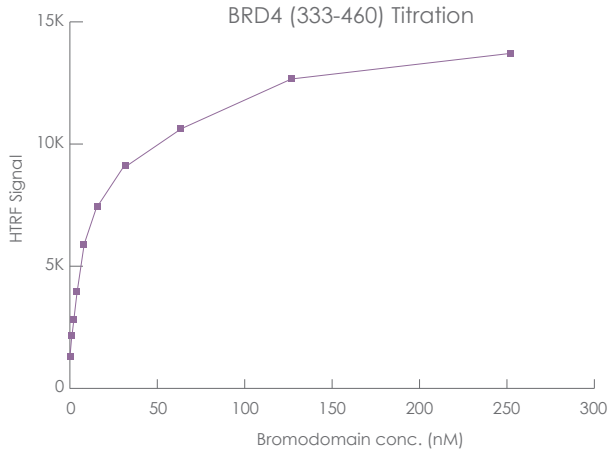


Figure 1: BRD4 (44-168) (top panel), and BRD4 (333-460) (bottom panel) bromodomains tested by HTRF. Assay conditions for bromodomain (BRD) activity were as follows: 3.3 µM histone peptide H4K5/8/12/16(tetra-acetyl) was incubated with the protein indicated in reaction buffer containing 50 mM HEPES-NaOH pH 7.0, 0.1% BSA for 1 hour at room temperature. Anti-FLAG antibody was used to detect the reaction products.

For a complete list of available purified recombinant proteins, please visit us at www.activemotif.com/proteins.

Methyltransferases and Demethylases

active, recombinant HMT and HDM proteins

One of the current challenges in the field of epigenetics research and drug discovery is developing robust enzymatic assays. The key to achieving a robust enzymatic assay is the ability to obtain high quality proteins.

Active Motif has produced over 60 methyltransferases and demethylases as well as several enzymatic complexes for many relevant drug targets, including NSD2, DOT1L, LSD1, KDM5A, KDM5B, KDM6A, and PRC2 and MLL complexes. The collection also includes N-terminal FLAG-tagged versions of HMTs and HDMs as well as a number of DNA methyltransferases (DNMTs).

Our HMTs and HDMs are manufactured to meet the highest standards of purity and activity required for drug discovery assays including fluorography, mass spectrometry, AlphaLISA and TR-FRET. In addition, dose response assessments performed with various reference compounds ensure utility of the enzymes in inhibitor studies.

MALDI-TOF Analysis Reveals High Level of Demethylase Activity of KDM5A / JARID1A Recombinant Protein

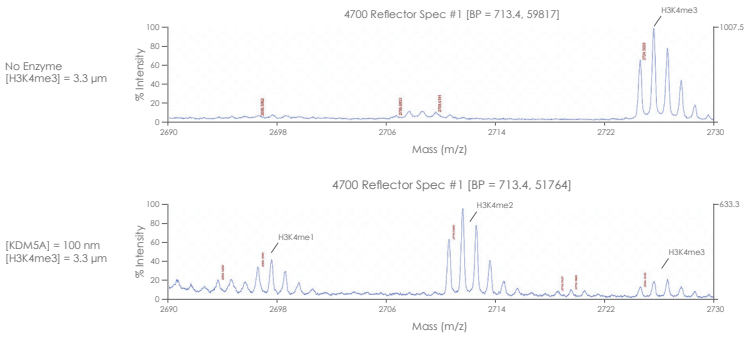


Figure 1: Mass spectrometry data demonstrate that our recombinant KDM5A enzyme is highly robust, requiring extremely low nM range amounts of protein to achieve high catalytic rates. The data show that only 100 nM KDM5A is sufficient to convert 65% 3.3 µM H3K4me3 peptides to H3K4me2 and 25% 3.3 µM H3K4me2 peptides to H3K4me1 in 1 hour. The catalytic rate of KDM5A: 40 turnovers/enzyme molecule/hour.

Activity Test for KDM5A using TR-FRET

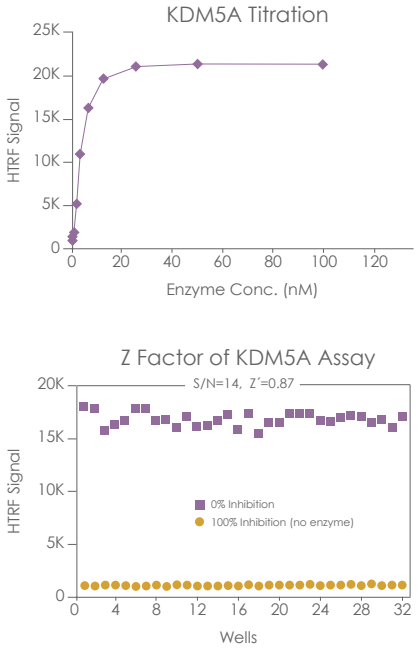


Figure 2: Date generated for the development of an HTRF (Homogeneous Time Resolved Fluorescence) assay in cooperation with Cisbio. HTRF combines fluorescence resonance energy transfer technology (FRET) with time-resolved measurement (TR) and is the most common assay used in drug discovery for high-throughput screening (HTS) of drug targets. Titration curve (left) was generated to show signal response in the presence of modified peptide substrate at increasing protein concentrations. The Z-factor assessment (right) was performed to quantify. the suitability of assay for use in a full-scale, high-throughput screen.

SPECIFICALLY DESIGNED FOR EPIGENETIC DRUG DISCOVERY

- Custom & bulk orders available
- Tagged versions available
- Lot specific data, including:
  - activity by HTRF and AlphaLISA
  - purity by SDS-PAGE and mass spec

Product	Catalog No.
DOT1L (1-416)	31474
EZH2 Complex	31337
JARID1A / KDM5A	31431
JARID1B / KDM5B	31432
LSD1 / KDM1A	31426
MLL1 Complex	31423
NSD1-SET	31475
PRC2 EZH2 (Y641C) Complex	31389
PRMT5	31393
SET2	31358
SMYD2	31497

For a complete list of available purified recombinant proteins, please visit us at www.activemotif.com/proteins.

HATs and HDACS

acetyltransferases and deacetylases

HATs and HDACs play an important role in gene regulation. Changes in acetylation patterns are observed between normal cells and cancer cells. In recent years, histone acetylases and deacetylases (HATS & HDACs) have emerged as important drug target candidates.

Active motif offers a large selection of HAT and HDAC proteins (full length and catalytic domains) as well as antibodies, inhibitors and fully validated kits for use in drug screening assays. Our recombinant proteins are manufactured to meet the highest standards of activity (Figure 1) and purity (Figure 2) required for conventional drug discovery assays.

14 HDACs AND HATS

- Full-length proteins and protein complexes
- Highly active
- Purity by SDS-PAGE
- Custom and bulk orders available

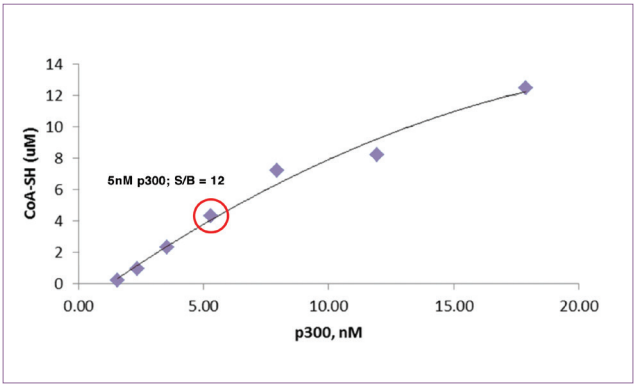


Figure 1: Recombinant p300 (1041-1161) activity assay. 3.3 µM histone peptide H4K5/8/12/16(4Ac) was incubated with p300 (1041-1161) protein in reaction buffer including 50 mM HEPES-NaOH pH 7.0, 0.1% BSA for 1 hour at room temperature. Anti-FLAG antibody was used to detect reaction products.

Purity by SDS-PAGE

HATs & HDACs

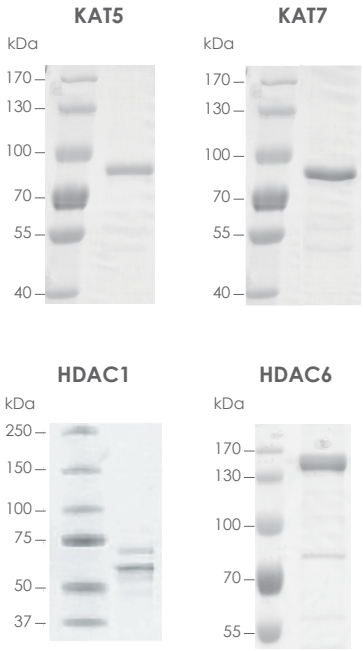


Figure 2: Full-length recombinant proteins and protein complexes were expressed and purified. Purified recombinant proteins were run on an SDS-PAGE gel and stained with Coomassie blue. The Coomassie stains reveal that recovered proteins are full length with little or no detectable degradation and were determined to have ≥ than 98% purity.

Product	Catalog No.
HDAC1	31342
HDAC3 Complex	31349
HDAC4	31350
HDAC5-catalytic domain	31351
HDAC6	31346
HDAC7	31352
HDAC8	31353
HDAC9	31354
p300	31124
SIRT1	31340
SIRT6	31336

IN-HOUSE MANUFACTURED AND QC'D

- Custom & bulk orders available
- Please contact us at www.activemotif.com/epiproteins

For a complete list of available purified recombinant proteins, please visit us at www.activemotif.com/proteins.

DNA Methylation Proteins

DNA methyltransferases and TET family

Changes in the methylation state have been implicated in many malignancies. As a result, DNA methyltransferase enzymes (DNMTs) have emerged as attractive therapeutic targets. Several therapeutic agents that are DNMT inhibitors have already been identified and there are many active programs searching for new inhibitory molecules. Other proteins involved in the regulation of DNA methylation include the TET family of proteins which have emerged as potential druggable targets.

Active Motif offers a range of DNA methylation-related proteins for use in drug screening including DNMTs and TET proteins. Our recombinant proteins are manufactured to meet the highest standards of purity and activity required for conventional drug discovery assays. In addition we offer a range of validated antibodies to proteins involved in the methylation process as well as against methylcytosine variants such as 5-mC, 5-hmC, 5-fC and 5-caC.

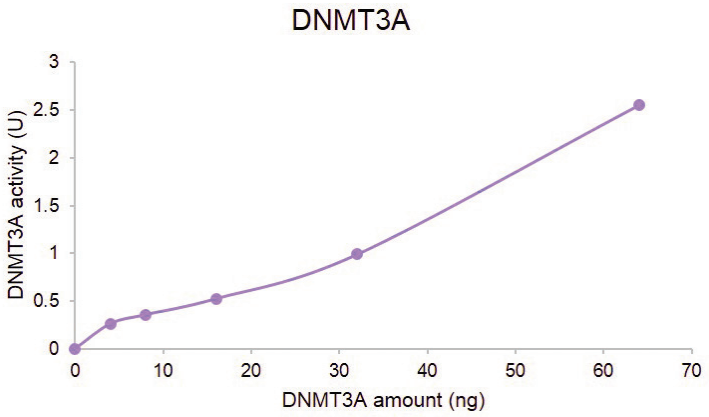


Figure 1: Recombinant DNMT3A protein activity assay. Recombinant DNMT3A protein activity was measured using the Active Motif DNMT Activity / Inhibition Assay (Catalog No. 55006), for 1 hour at 37°C.

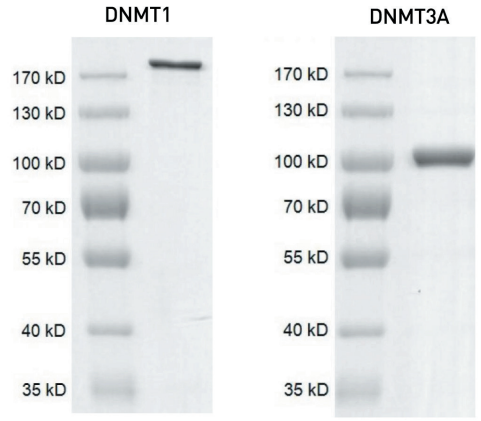


Figure 2: SF9 expressed DNMT proteins. Coomassie staining reveals that recovered proteins are full-length with little or no detectable degradation and determined to have greater than or equal to 98% purity.

Product	Catalog No.
DNMT1	31404
DNMT3A	31406
DNMT3A / DNMT3L Complex	31415
DNMT3B	31413
DNMT3B / DNMT3L Complex	31416
DNMT3L	31414
TET1 (1418-2136)	31417
TET2 (1129-2002)	31418
TET3 (824-1795)	31421

ACTIVE MOTIF DNA METHYLATION PROTEINS

- Specifically designed for epigenetic drug discovery
- Lot specific data, including:
  - activity data
  - purity by SDS-PAGE

For a complete list of available purified recombinant proteins, please visit us at www.activemotif.com/proteins.

Substrates For Epigenetic Enzymes

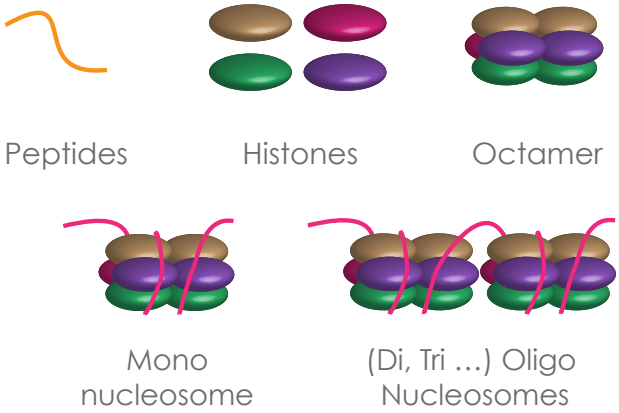
physiologically relevant substrates for biochemical assays

A challenge associated with epigenetic screening assays is the choice of substrate. The biological targets of the epigenetic enzymes within the cell are not free histones. Rather, histone targets are organized into nucleosomes that are further packaged into high order structures within chromatin. The complexity and structural variability of chromatin represents a unique drug discovery challenge for these classes of enzymes because it is difficult to reproduce the chromatin structure *in vitro*. Therefore, alternative substrates, such as short modified peptides that can effectively mimic the histone tail, are often used in high-throughput screens.

However, peptides do not reproduce the complexity of the native biological substrate and do not always interact with epigenetic enzymes in a way that yields a productive reaction. Other types of substrates have been shown to perform better with specific enzymes and include modified or unmodified full-length histones, histone octamers, mononucleosomes and oligonucleosomes (Figure 1). Therefore, critical decisions need to be made during assay development with regards to what substrate choice will perform best with its associated enzyme in the assay.

LARGEST COLLECTION OF SUBSTRATES

- Nucleosomes
- Unmodified and modified histones
- Histone octamers
- Biotinylated substrates



IN-HOUSE MANUFACTURED AND QC'D

- Custom & bulk orders available
- Please contact us at www.activemotif.com/epiproteins

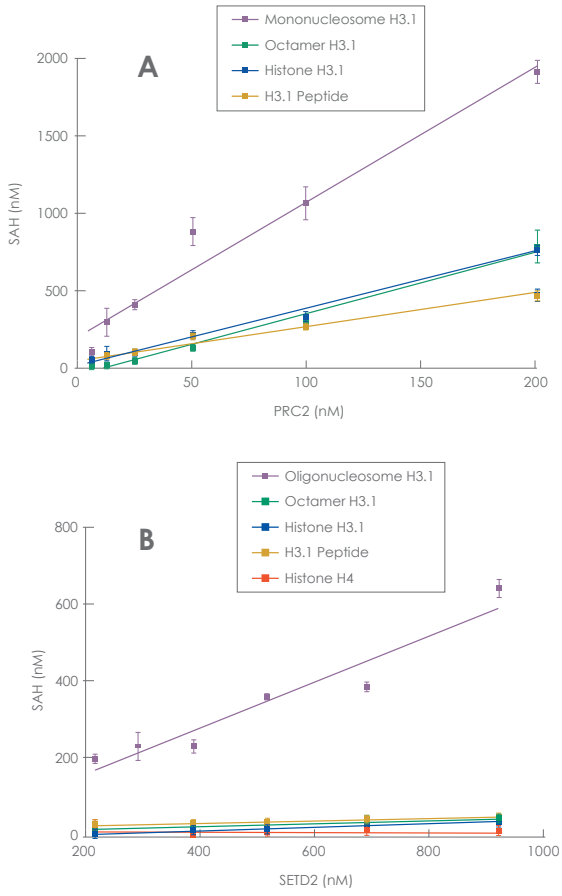


Figure A: PCR2 favors mononucleosomes over other substrate alternatives. Activity of PRC2, a complex of EZH2, EED, SUZ12 and RbAp46/48, towards a variety of substrates was measured using an HTRF assay detecting conversion of SAM to SAH.

Figure B: SETD2 has an absolute requirement for nucleosome substrates. Comparison of SETD2 activity towards a variety of substrates measured using an HTRF assay.

For a complete list of available substrates, please visit us at www.activemotif.com/proteins.

Full-Length Histones

unmodified & modified histone substrates

Active Motif offers an exclusive collection of over 60 full-length unmodified and modified recombinant histones, including site- and degree-specific modifications such as methylation, acetylation and phosphorylation. Because of our patented Expressed Protein Ligation (EPL) and Methylated Lysine Analog (MLA) synthesis technologies, Active Motif is the exclusive source of most of our full-length modified histones.

The outcome of your *in vitro* biochemical assay is more accurate when the system mimics actual cellular biology. Use our histone proteins as stand-alone substrates or assemble to generate nucleosomes and oligonucleosomes. Alternatively, save time and effort by choosing from our selection of pre-assembled human histone octamers and nucleosomes (see opposite page).

THE LARGEST COLLECTION OF RECOMBINANT HISTONES AVAILABLE

- Over 60 recombinant histones
- Site- and degree-specific modifications
- Patented synthesis technologies
- Biotinylated histones available for FRET

WHICH HISTONE SUBSTRATE IS RIGHT FOR YOUR ASSAY?

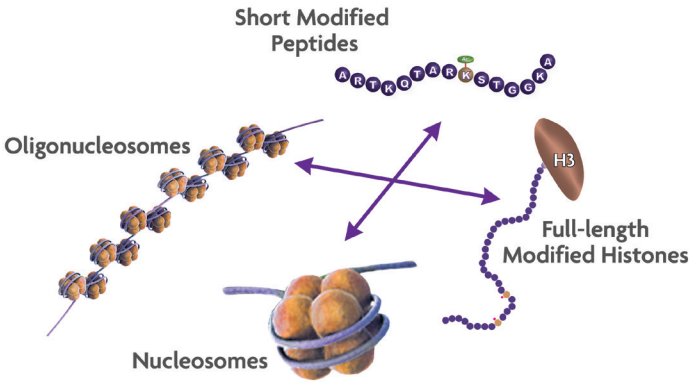


Figure: Choosing the correct histone substrate for assay development is key to achieving the best results. Opinion leaders in epigenetics recognize the power of reconstituting recombinant chromatin for creating biologically relevant substrates.

Product	Catalog No.
Histone H3.1 biotinylated	31296
Histone H2A	31490
Histone H2B	31492
Histone H4	31223
Histone H3K4me1	31208
Histone H3K4me2	31277
Histone H3K4me3	31278
Histone H3K9me1	31281
Histone H3K9me2	31280
Histone H3K9me3	31279
Histone H3K9ac	31253
Histone H3K14ac	31254
Histone H3K18ac	31273
Histone H3K27ac	31290
Histone H3K27me3	31216
Histone H3K36me2	31218
Histone H3K79me2	31221
Histone H4K20me1	31224

For a complete list of available recombinant histones, please visit us at www.activemotif.com/recombhis.

Nucleosomes and Histone Octamers

physiologically relevant assay substrates

Whether performing drug screens, analyzing enzyme kinetics or monitoring changes in histone modifications, substrate selection is critical.

Pre-assembled histone octamers and nucleosomes better mimic cellular biology when compared to histone proteins alone or synthetic peptides.

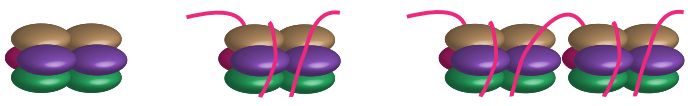
ADVANTAGES

- More physiologically relevant
- Improves enzyme activity
- Better assay specificity
- Biotinylated versions available

In vivo, histones are assembled into octamers and are wound by DNA in chromatin, making octamers and nucleosomes more physiologically relevant substrates than histones and histone-derived peptides for *in vitro* studies. More importantly, some histone methyltransferases (HMT), such as DOT1L and NSD enzymes, are significantly more active and specific when using nucleosome substrates in HMT assays. Nucleosomes are also widely used in HMT screening assays to identify small molecule inhibitors for drug discovery.

No assembly required

Active Motif's recombinant histone octamers and nucleosomes come pre-assembled to save you time and money. Histone octamers are comprised of unmodified core histone proteins (H2A, H2B, H3 and H4). For nucleosomes, the histone octamer is bound by DNA. Both unlabeled and biotin-labeled versions are available to enable flexibility in your experimental design.



Octamer | Mono nucleosome | (Di, Tri …) Oligo Nucleosomes

ACHIEVE BETTER RESULTS WITH THE RIGHT CHOICE OF SUBSTRATE

1. No enzyme
2. No substrate
3. Histone Octamers
4. Nucleosomes A
5. Nucleosomes B

Figure 1: To illustrate the importance of choosing the correct substrate to achieve the best results, recombinant nucleosomes were compared with histone octamers in a histone methyltransferase assay using SET domain-containing HMT enzymes MMSET/NSD1-SET and NSD2-SET. The results show there is greater methyltransferase activity when using recombinant nucleosomes as substrates when compared to octamers alone.

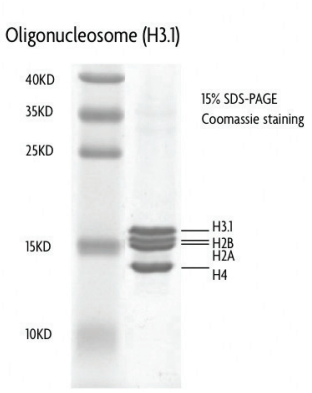


Figure 2: Recombinant nucleosomes (H3.1) run on an SDS-PAGE gel and stained with Coomassie blue.

Product	Catalog No.
Histone Octamer (H3.1)	31470
Histone Octamer (H3.1) - biotinylated	31471
Histone Octamer (H3.3)	31472
Histone Octamer (H3.3) - biotinylated	31473
Nucleosomes (H3.1)	31466
Nucleosomes (H3.1) - biotinylated	31467
Nucleosomes (H3.3)	31468
Mononucleosomes (H3.3) - biotinylated	31469
Nucleosome Preparation Kit	53504
Chromatin Assembly Kit	53500
Nucleosome Assembly Control DNA	53502

For a complete list of available substrates, please visit us at www.activemotif.com/proteins.

Small Molecules

to modulate activity of epigenetic enzymes

Active Motif offers an expanding collection of small molecule compounds (activators and inhibitors) to modulate the activity of proteins that regulate DNA methylation, chromatin and histones.

These compounds are ready for use as epigenetic drug discovery tools for lead generation and assay development. Our comprehensive selection includes compounds that target DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases (HDMs), and the BET family of bromodomains. These products can be readily scaled to adapt to the requirements of your research.

Description	Target	Cat. No.
LYSINE DEMETHYLASE AND METHYLTRANSFERASE		
AS-8351	KDM5B inhibitor	14112
BIX-01294	G9a inhibitor	14072
Daminozide	KDM2/KDM7 inhibitor	14058
DMOG	PHD/JMJD2A inhibitor	14062
GSK-J1 (cell impermeable)	JMJD3/UTX inhibitor	14068
GSK-J4 (cell permeable)	JMJD3/UTX inhibitor	14070
IOX1	JMJD family inhibitor	14056
ML-324	JMJ2 inhibitor	14078
PBIT	JARID1 inhibitor	14110
Tranylcypromine hemisulfate	LSD1/BHC110 inhibitor	14046
Chaetocin	Su(VAR)3-9 inhibitor	14051
NSC-663284	SETD8 inhibitor	14122
UNC-1999	EZH2 inhibitor	14114
HISTONE ACETYLTRANSFERASE		
C646	p300/CBP inhibitor	14052
CPTH2	GCN5P inhibitor	14094
CTPB	p300 activator	14064
Embelin	PCAF inhibitor	14118
Garcinol	p300/PCAF inhibitor	14076
HISTONE DEACETYLASE		
Apicidin	HDAC inhibitor	14040
BML-210	HDAC inhibitor	14048
CI-994	HDAC inhibitor	14092
CUDC-101	HDAC & receptor tyrosine kinase inhibitor	14060
HPA (Hexyl-4-pentynoic acid)	HDAC inhibitor	14034
MS-275	HDAC inhibitor	14042
Panobinostat	Class I & II HDAC inhib.	14044
Phenylbutyrate Na	HDAC inhibitor	14033
Romidepsin	HDAC inhibitor	14083
TM-2-51	HDAC8 activator	14096
Trichostatin A	HDAC inhibitor	14038
Tubastatin A hydrochloride	HDAC6 inhibitor	14084
Valproic acid, sodium salt	HDAC inhibitor	14021
Vorinostat (SAHA)	HDAC inhibitor	14026

Description	Target	Cat. No.
DNA METHYLATION		
2´-Deoxy-5-fluorocytidine	DNMT inhibitor	14108
Decitabine (5-Aza-2´-deoxycytidine)	DNMT inhibitor	14100
5-Azacytidine	DNMT inhibitor	14102
6-Thioguanine	Degradation of DNMT	14126
Mithramycin A	DNMT1 inhibitor	14128
RG108	Non-nucleoside DNMT inhibitor	14104
Zebularine	DNMT inhibitor	14106
SIRTUIN		
AK-7	SIRT2 inhibitor	14054
BML-278	SIRT1 activator	14024
EX-527	SIRT1 inhibitor	14028
Piceatannol	SIRT1 activator	14036
Resveratrol	SIRT1 activator	14022
Salermide	SIRT1/SIRT2 inhibitor	14124
SirtAct	SIRT1 activator	14080
Sirtinol	Sirtuin inhibitor	14074
Splitomicin	Sir2p (yeast Sirt1 homolog) inhibitor	14086
Triacetyl-resveratrol	Sirtuin activator	14116
BROMODOMAIN		
JQ1 (racemic)	BET bromodomain inhibitor	14066
RVX-208	BET bromodomain antagonist	14090
ARGININE METHYLTRANSFERASE		
TC-E-5003	PRMT1 inhibitor	14099
OTHER		
Sinefungin	Methyltransferase (DNA, RNA, protein) inhibitor	14088
UNC-2170	Methyl-lysine binding protein inhibitor	14120

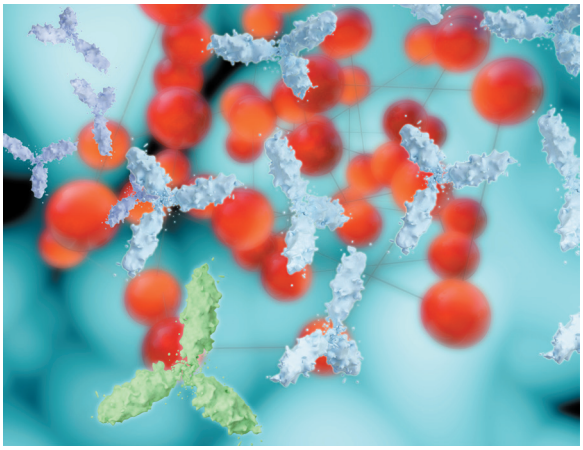
For a complete list of available activators and inhibitors, please visit us at www.activemotif.com/smallmol.

Antibodies

for discovery & analysis of druggable targets

Active Motif is more than just an antibody supplier. We identify, manufacture and rigorously test each antibody in our vast collection to ensure that we offer only the highest quality product.

We monitor every step from immunogen design and specificity screening to application validation. This thorough process ensures our antibodies meet the high standards for consistency and quality you require to achieve the goals of your drug discovery and development efforts.



ANTIBODIES FOR RELEVANT DISEASE AREAS

- Oncology
- Immunology
- Inflammation
- Neurobiology
- Metabolic disorders
- Cardiovascular disease

OVER 700 KEY DNA METHYLATION, HISTONE AND TRANSCRIPTION FACTOR TARGETS

EPIGENETICS

- H2A, H2B, H3 & H4 PTMs
- DNA methylation
- Bromodomain proteins
- Methyltransferases
- Demethylases
- Acetyltransferases
- Deacetylases

TRANSCRIPTION FACTORS

- Hormone receptors
- NFκB family
- STAT family
- IRF family
- AP-1 family
- p53
- Oct4

ADVANTAGES

- Rigorous application testing
- Assay-compatible formulations
- Large lot sizes
- Monoclonals to histone PTMs

NEW

- Recombinant antibodies to epigenetic marks
- Custom conversion of monoclonals
- Proprietary tag for enzymatic labeling and solid support attachment
- Please contact us for more information

Product	Catalog No.
BRD4 antibody (pAb)	39909
DOT1L antibody (pAb)	39953
EED antibody (mAb)	61203
EZH2 antibody (mAb)	39875
HDAC1 antibody (mAb)	39531
HDAC2 antibody (mAb)	39533
Histone H3K4me3 antibody (mAb)	61379
Histone H3K27me3 antibody (mAb)	61017
Histone H3K36me2 antibody (mAb)	61019
Histone H3K79me2 antibody (pAb)	39143
LSD1 / KDM1A antibody (pAb)	61607
MMSET / WHSC1 antibody (mAb)	39879
Smyd3 antibody (pAb)	61407

For more information and a complete list of available antibodies, please visit us at www.activemotif.com/abs.

Screen Binding Specificity

of antibodies, proteins & enzymes

The MODified™ Histone Peptide Array enables rapid, high-throughput screening of antibodies, enzymes and proteins for cross-reactivity or binding interactions with histones and their modifications. Each peptide array contains 384 unique histone modification combinations in duplicate, with up to four separate modifications on the same 19mer peptide.

- **Most extensive coverage of modifications on the market**
  384 unique combinations enable the study of not only individual sites, but also neighboring effects of modifications on recognition and binding.

- **Unique synthesis & conjugation methods**
  ensure >95% purity of bound peptides and high peptide density at each spot, which is advantageous for analysis of interaction sites with low binding constants.

- **Simple Western blot-like array protocol**
  no special equipment needed.

Two Array Labeling Kit versions are available:

- **MODified Array Labeling Kit**
  to study antibody and enzyme interactions.

- **MODified Protein Domain Binding Kit**
  to analyze the binding specificity of His-tagged protein reader domains.

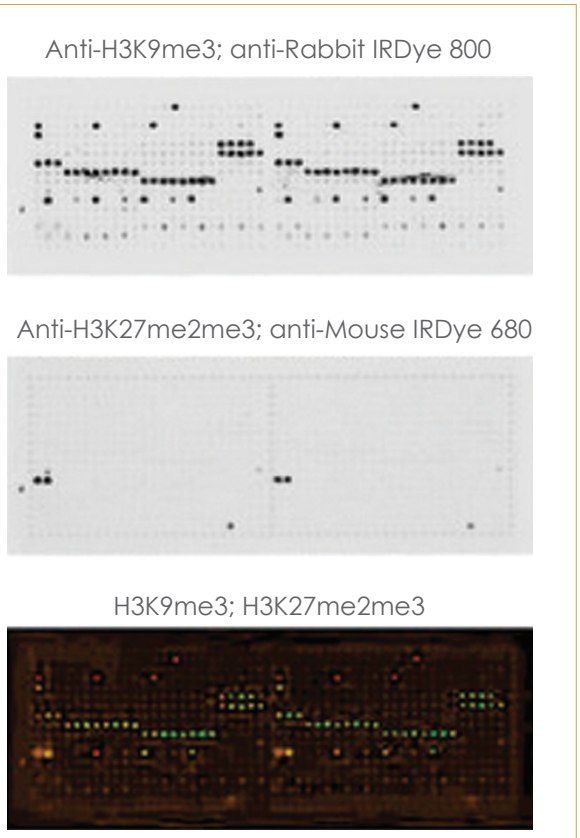


Figure: Analysis of the MODified Histone Peptide Array using the LI-COR Odyssey Infrared Imaging System. The top image shows the binding pattern of the H3K9me3 antibody alone, the middle image shows the binding pattern of the H3K27me2me3 antibody in combination with the c-Myc control antibody from the Array Labeling Kit, and the bottom image is the composite from the Odyssey system. The results of the LI-COR Odyssey Infrared Imaging system show that dual detection is possible with the MODified Histone Peptide Arrays.

Product	Catalog No.
MODified™ Histone Peptide Array, 1 array	13001
MODified™ Histone Peptide Array, 5 arrays	13005
MODified™ Array Labeling Kit	13006
MODified™ Protein Domain Binding Kit	13007

Application	Array	Labeling Kit
Antibody Specificity	MODified™ Histone Peptide Array	Array Labeling Kit
Enzymatic Effects	MODified™ Histone Peptide Array	Array Labeling Kit
Protein Domain Specificity	MODified™ Histone Peptide Array	Protein Domain Binding Kit

Table of MODified array reagents recommended for specific applications. The MODified Array Labeling Kit and the MODified Protein Domain Binding Kit are complementary products containing the buffers and reagents required to process the array for detection. The above table shows the combination of reagents recommended for each application.

For more information on the MODified Histone Peptide Array and Labeling Kits, please visit us at www.activemotif.com/modified.

Measure Global Changes in the Epigenome

generate histone profiles of your cell models

Active Motif offers a variety of high-throughput, quantitative, ELISA-based assays to measure global changes in histone modifications in response to drug treatment.

Screen the effects of compounds and other variables on histone modification levels

The addition or removal of modifications such as phospho, methyl and acetyl functional groups to histones have a profound effect on the regulation of transcription, chromosome packaging and DNA damage repair. Screening extracts for specific histone modifications is a simple way to assess cell health and compound effects.

Active Motif's comprehensive selection of Histone Modification ELISAs provides a simple solution for screening changes in histone H3 modification levels from purified core histones (see Product Ordering Information for a list of available Histone Purification Kits) or histones isolated by acid extraction.

HISTONE MODIFICATION ELISAS FOR:

- Total Histone
- H3K4me1
- H3K4me2
- H3K4me3
- H3K9me2
- H3K9me3
- H3K27me1
- H3K27me3
- H3K9ac
- H3K14ac
- H3S10ph
- H3S28ph

ASSAY FEATURES

- QUANTITATIVE – Includes control standards for easy and accurate quantitation
- THROUGHPUT – Stripwell microplate format for manual or high throughput analysis
- SIMPLE – ELISA based colorimetric assay
- SPECIFIC – tested for cross-reactivity with other degree-specific histone modifications

MEASURE CHANGES IN HISTONE MODIFICATIONS IN RESPONSE TO DRUGS

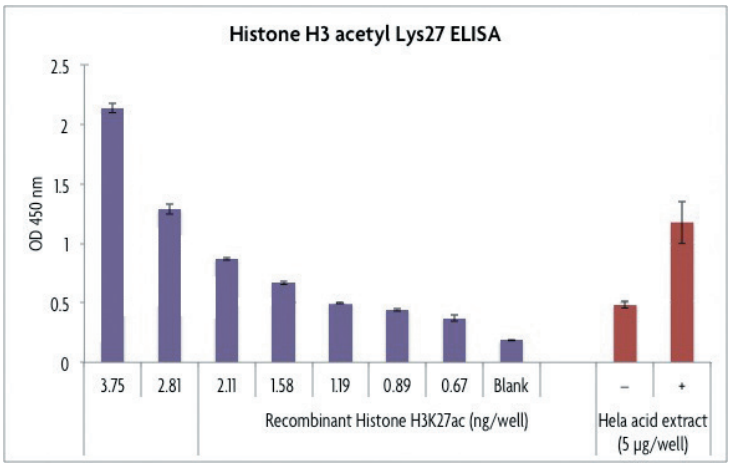


Figure: Histone H3 acetyl Lys27 (H3K27ac) ELISA. The Histone H3 acetyl Lys27 ELISA was used to assay HeLa acid extracts (5 µg) that were either untreated (–) or treated with sodium butyrate (+), a known HDAC inhibitor. The provided Recombinant Histone H3 acetyl Lys27 protein was assayed from 0.67 - 3.75 ng/well as a reference standard curve.

Product	Catalog No.
Total Histone H3 ELISA	53110
Histone H3 monomethyl Lys4 ELISA	53101
Histone H3 dimethyl Lys4 ELISA	53112
Histone H3 trimethyl Lys4 ELISA	53113
Histone H3 acetyl Lys9 ELISA	53114
Histone H3 dimethyl Lys9 ELISA	53108
Histone H3 trimethyl Lys9 ELISA	53109
Histone H3 phospho Ser10 ELISA	53111
Histone H3 acetyl Lys14 ELISA	53115
Histone H3 monomethyl Lys27 ELISA	53104
Histone H3 trimethyl Lys27 ELISA	53106
Histone H3 phospho Ser28 ELISA	53100
Histone Purification Kit	40025
Histone Purification Mini Kit	40026
Histone Purification Microplate Kit	40027

To learn more about our histone analysis products, visit www.activemotif.com/hismodinfo.

Measure Global Changes in the Epigenome

assay specific & off-target effects in a single well

Active Motif has developed the first multiplex epigenetic assay for studying histone post-translational modifications (PTMs). The Histone H3 PTM Multiplex Assay is designed for use with Luminex instruments, enabling high-throughput processing of nanogram-sized samples to simultaneously interrogate multiple PTMs in a single reaction.

Time-consuming Western blots can now be replaced in early target validation programs that correlate global histone modification enrichment or depletion with disease. The Histone H3 PTM Multiplex Assays provide a rapid, highly sensitive, high-throughput method for profiling specific and off-target changes in histone PTMs in a multiplex format. Get more data from less input, in less time and at a lower cost.

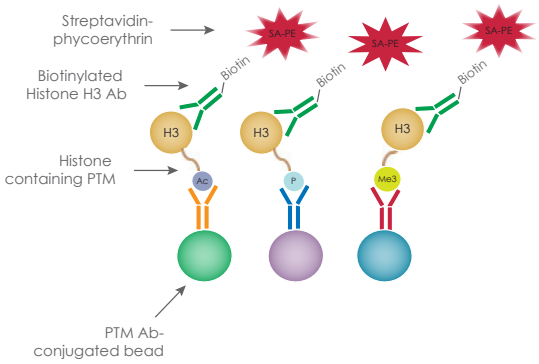


Figure1: Schematic of the Histone H3 PTM Multiplex Assay. The assay works as a solution-based sandwich ELISA to evaluate histone H3 PTM levels.

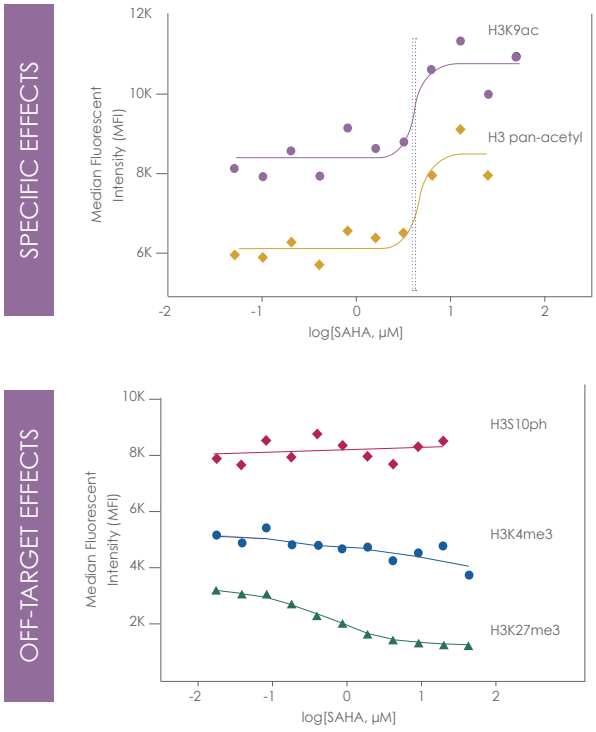


Figure 2: The Histone H3 PTM Multiplex Assay shows increased histone acetylation in response to SAHA-mediated HDAC inhibition. HeLa cells pre-treated with increasing concentrations of the HDAC inhibitor SAHA were evaluated in a multiplex of H3 pan-acetyl, H3S10ph, H3K9ac, H3K4me3, H3K27me2 & H3 Total Ab-conjugated beads using the Histone H3 PTM Multiplex Assay. The results demonstrate the ability of the assay to simultaneously assess specific and off-target effects of the treatment on histone modification levels. The dashed lines represent IC_{50} values determined for the acetyl marks.

ASSAY FEATURES

- MULTIPLEX – the only kit available to perform multiplexed histone modification analysis
- EFFICIENT – use less input & less time than WB to assay multiple histone PTMs
- SENSITIVE – requires only nanogram amounts of crude acid extracts or purified histones
- HIGH CONTENT – simultaneously compare specific and off-target effects in a single reaction

To learn more about our Luminex assays, visit us at www.activemotif.com/luminex.

Custom Services

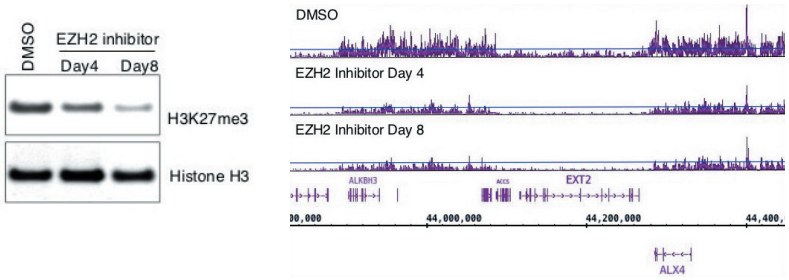
epigenetic and gene regulation services

Active Motif offers a suite of services specifically designed to enhance drug development programs, especially those focused on small molecule epigenetic inhibitors. In the early phases of drug discovery our recombinant proteins and cell-based screening services will accelerate your program, while in the late phase our genomic assays, such as ChIP-Seq, RNA-Seq, MeDIP-Seq and Open chromatin-Seq will lead to a greater understanding of your compound's mechanism of action.

TAKE ADVANTAGE OF OUR EXPERTISE

- Identify mechanism of action at the epigenetic level
- Characterize inhibitors of epigenetic targets
- Determine the MOA and off-target effects of your lead compounds

IDENTIFY MECHANISM OF ACTION AT THE EPIGENETIC LEVEL



Figures: A) H3K27me3 Western blot shows a reduction in global H3K27me3 levels after EZH2 inhibition. B) ChIP-Seq data shows reduced H3K27me3 levels after exposure to an EZH2 inhibitor for 4 and 8 days. The decrease in H3K27me3 signal is only revealed by using Active Motif's ChIP-Seq spike-in normalization protocols.

EPIGENETICS

- RNA-Seq
- ChIP-Seq
- OPEN CHROMATIN-Seq
- INTERACTOME PROFILING
- HISTONE PTM QUANTITATION
- DNA METHYLATION
- BIOINFORMATIC SOLUTIONS

GENE REGULATION

- CELL-BASED PATHWAY ANALYSIS
- miRNA FUNCTIONAL ANALYSIS
- REPORTER ASSAY SCREENS
- SEQUENCE VARIANT SERVICES

To view the full range of services and for more information, please visit us at www.activemotif.com/services.

Gene Expression Services

services for whole transcriptome sequencing and gene expression analysis

Active Motif's transcriptome analysis services include RNA-Seq for identification and quantitation of RNA transcripts as well as 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-SEQ SERVICES

Simply submit RNA, cell 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
- Phenotypic effects of TF binding on gene expression

RNA-SEQ SERVICES FEATURES

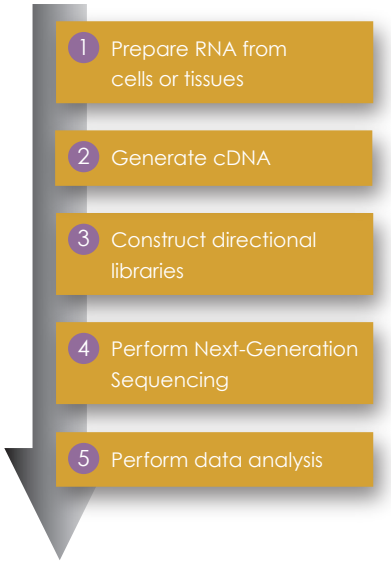
- PolyA selection or rRNA reduction
- QC performed using Bioanalyzer
- Directional library preparation
- 50bp or 100bp paired-end reads
- Data analysis options (TOPHAT, CUFFLINKS, CUFFCOMPARE, CUFFDIFF)

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

RNA-SEQ SERVICES



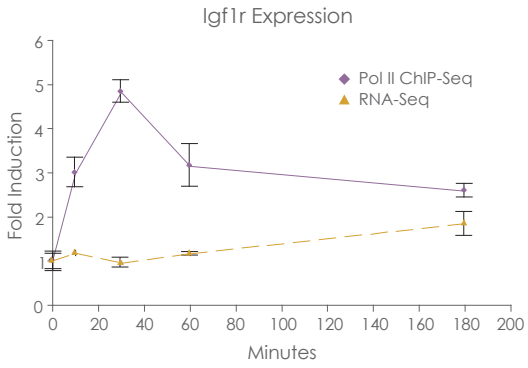


Figure: Gene expression profiles vary depending on the analysis method. Data for Igf1r was extracted from RNA–Seq and RNA Pol II ChIP-Seq data sets. Cell treatment resulted in induced gene expression that was measured at various time points. The cumulative data show that, as measured by RNA Pol II ChIP-Seq, transcription is induced immediately, while mRNA levels only accumulate over time.

To view the full range of services and for more information, please visit us at www.activemotif.com/services.

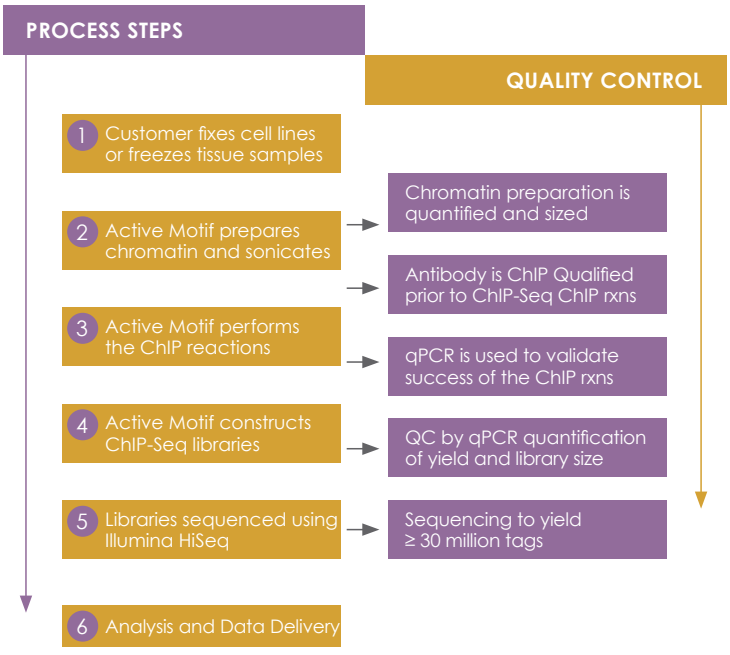
ChIP-Seq Services

end-to-end ChIP-Seq services

Targeting epigenetic modifying enzymes for drug development has great potential for advancing research in the fields of cancer, inflammation, development and neurobiology. The in-depth characterization of lead compounds in these areas requires a genome-wide mechanistic perspective of how these compounds alter epigenetic profiles.

Active Motif's Epigenetic Services team specializes in genome-wide compound characterization and offers this assay as an end-to-end ChIP-Seq service to accelerate your drug discovery and development program.

CHIP-SEQ SERVICES



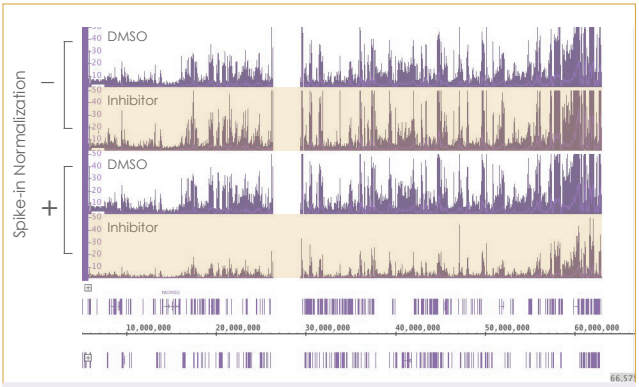


Figure: ChIP-Seq Spike-in Normalization Strategy reveals changes in H3K27me3 levels following treatment with EZH2 inhibitor compound. Cells treated with a small molecule inhibitor of EZH2 methyltransferase have dramatic reductions in global H3K27me3 levels. However, H3K27me3 ChIP-Seq using standard ChIP-Seq protocols (-) does not detect these differences. Incorporation of Active Motif's ChIP-Seq Spike-in Strategy (+) reveals the expected decrease in H3K27me3 ChIP-Seq signal.

NEW: ChIP-Seq Spike-in Strategy

Traditional ChIP-Seq protocols do not always detect global changes in histone modifications following treatment with epigenetic inhibitors. Our novel ChIP-Seq spike-in and normalization strategy is able to reveal these differences and is now offered as part of our end-to-end ChIP-Seq Service.

- *Drosophila* chromatin is "spiked in" to a ChIP reaction
- A second *Drosophila*-specific antibody is added to reliably pull down a small fraction of *Drosophila* chromatin
- *Drosophila* tag counts are equalized across samples and used to normalize ChIP-Seq data

RELATED SERVICES

Active Motif also offers several other end-to-end whole-genome services that can be combined to meet your specific research needs.

- Open Chromatin-Seq – identify open regions
- Low Cell ChIP-Seq – as low as 50,000 cells
- RRBS – profile genome-wide DNA methylation

VALIDATED ASSAYS FOR OVER 200 TARGETS

- BRD4
- EZH2
- LSD1
- H3K9ac
- H3K27me3
- H3K4me2

To view the full range of services and for more information, please visit us at www.activemotif.com/services.

Interactome Profiling

IP-mass spectrometry service for identification and characterization of protein interactomes

The newest addition to Active Motif's suite of custom services, RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins) sheds light on the complex process of gene regulation by enabling the capture and identification of interactomes, or the associated protein networks, of an endogenous protein of interest.

Identify interactomes regulating gene expression

Gene regulation is often oversimplified when the focus is on one particular transcription factor (TF) in any given cell model. In reality, differential gene expression is greatly influenced by cofactors and other protein interactions with chromatin. RIME sorts out this complexity by providing a means to identify the protein interactions that are important for gene regulation.

Why RIME?

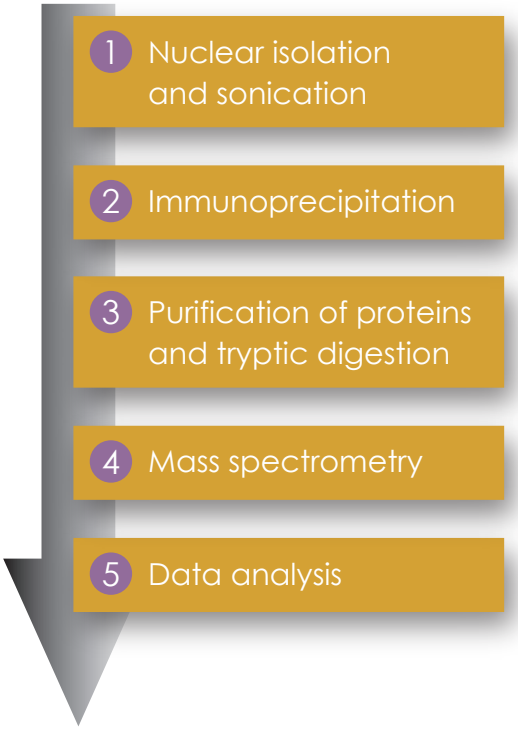
Immunoprecipitation (IP) followed by mass spectrometry has traditionally been the approach used to identify proteins interacting with a target of interest.

RIME vs traditional IP

RIME differs from traditional IP approaches because the starting material has been cross-linked, offering the advantage of:

1) targeting only DNA, chromatin and associated proteins,
2) enabling capture of low affinity interactions which are lost in IP/mass spectrometry,
3) allowing more stringent wash conditions resulting in less non-specific interactions, and
4) stabilizing protein-DNA interactions to enable the capture of proteins that are bound at adjacent sites in the DNA and are independent of protein-protein interactions.

RIME SERVICES



RIME ADVANTAGES

- Identify transcriptional cofactors
- Identify TFs bound to DNA at sites adjacent to your target TF
- Identify important targets for ChIP-Seq analysis
- 50bp or 100bp paired-end reads
- Detect low affinity interacting proteins

To view the full range of services and for more information, please visit us at www.activemotif.com/services.

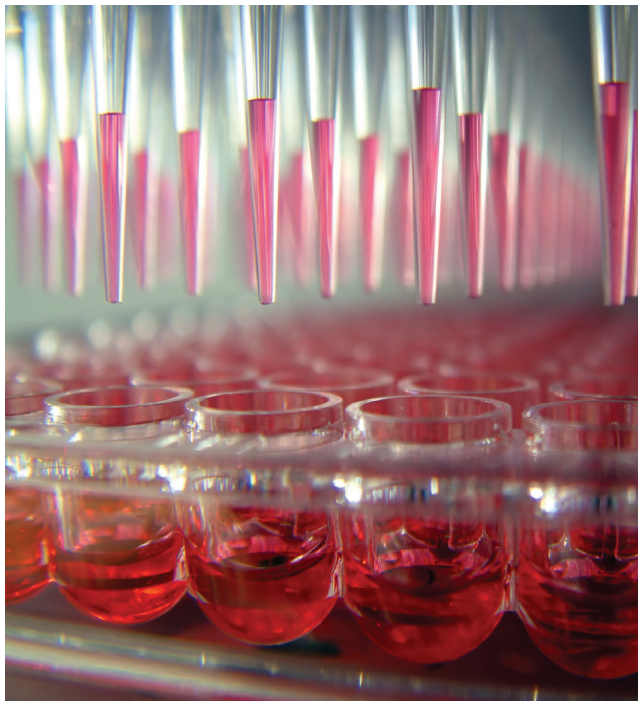
Cell-Based Pathway Analysis

identify biological responses to compound treatment

In understanding a compound's mechanism of action it is crucial to identify activation of both intended and unintended cellular pathways. LightSwitch™ Pathway Screening Assays help you characterize cellular responses to compound treatment and understand off-target effects.

Our unique collection of validated promoter reporters measure gene expression changes associated with a number of disease-related biological pathways. Our standard LightSwitch Pathway Screening Assays are available as stable cell lines or transfection-ready plasmids. Custom pathway sets are also available.

- Analyze 15 pathways in an HTS environment
- Identify compound-mediated pathway activation
- Determine the MOA and off-target effects of your lead compounds
- Available as a product or a service



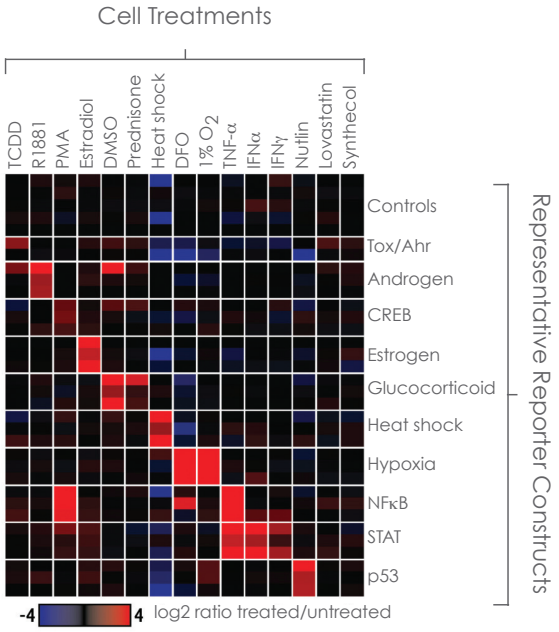


Figure 1: LightSwitch Pathway Screening Services can profile pathway responses to treatments. The heat map above shows the inducible activity of 29 different reporter constructs that represent 10 different pathways in response to 15 different treatments.

PATHWAY PROFILING PANEL

48 promoters and controls

Pathway activity readout for:	
HIF-1α	Hypoxia
NFκB	Inflammation
CREB	cyclic AMP
HSF1	Heat shock
p53	DNA damage, apoptosis
STAT	Interferon
SREBP	Cholesterol biosynthesis
ER	Estrogen
AR	Androgen
GR	Glucocorticoid
AhR	Toxicity

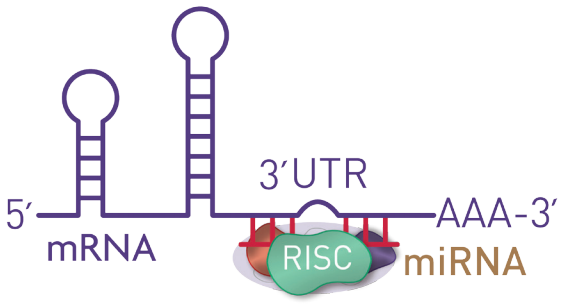
To learn more about our LightSwitch Pathway Screening Assays and Services, please visit us at www.activemotif.com/lightswitch.

Functional Assays for miRNA Research

measure miRNA function and validate miRNA targets

MicroRNAs (miRNAs) are involved in many areas of disease biology, and are actively being tested in the clinic as drug targets and therapeutic molecules. The LightSwitch™ 3´UTR Reporter Assay provides an HTS system that is ideally suited for screening the effects of small molecules and chemical modifications on miRNAs.

The LightSwitch 3´UTR Reporter GoClone™ Collection includes over 12,000 ready-to-transfect human 3´UTR reporter vectors and 1000 synthetic miRNA target reporters to study miRNA-3´UTR interactions, validate miRNA targets, and measure RNA stability, translation efficiency and the functional impact of miRNAs on a gene-by-gene basis.



Understanding the biological role of a miRNA often requires identification of downstream targets. The LightSwitch 3´UTR reporter system is a validated assay that can rapidly identify hundreds of miRNA target genes in a single experiment.

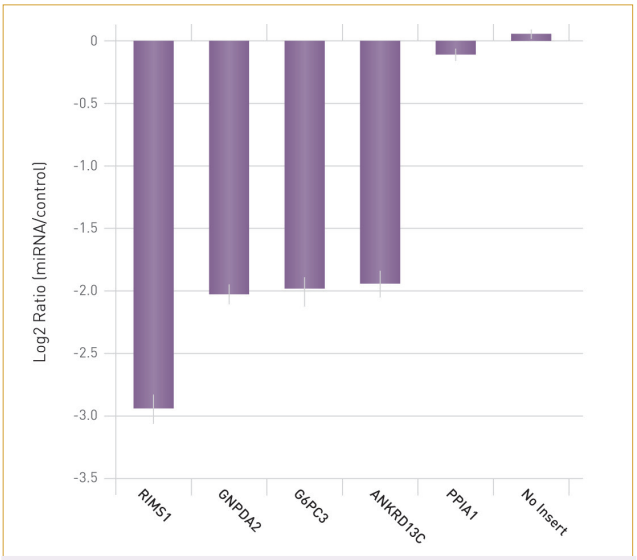


Figure: Interaction of miR-122 with 3´UTR targets. 3´UTR targets of miR-122 were cloned into the LightSwitch 3´UTR vector and co-transfected with either miR-122 mimic or a non-targeting control miRNA into K-562 cells. The graph shows the four strongest responders along with two non-responding controls.

- Measure the effects of small molecules or chemical modifications on miRNA activity
- Validate downstream miRNA targets
- Identify all miRNAs that regulate a gene of interest
- miRNA screening services also available
- Determine seed sequence variant effects

PRODUCTS FOR miRNA FUNCTIONAL SCREENS

- 12,000 pre-cloned human 3´UTR reporters
- Synthetic target reporters containing sequences fully complementary to 1000 human miRNAs
- miRNA mimics and inhibitors
- Validated 3´UTR controls
- Complete screening service to validate 3´UTR targets for any miRNA

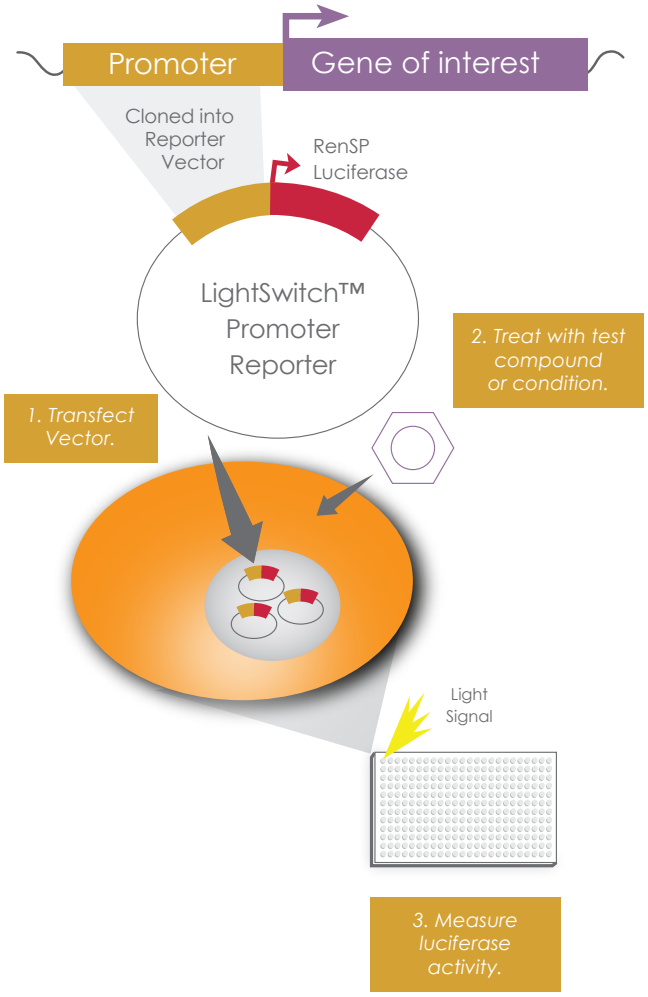
For more complete information on our miRNA targets, mimics, inhibitors and services, please visit www.activemotif.com/ls-3utr.

Reporter Assays for Drug Screening

accelerate reporter assay screens with the LightSwitch™ System

The LightSwitch™ Luciferase Assay System dramatically reduces the time investment needed to identify the most responsive promoter to develop as a new HTS reporter assay. Over 18,000 pre-cloned human promoter reporter vectors are available to choose from for immediate incorporation into plate-based screens.

- 18,000 pre-cloned human promoter reporters for screening expression changes of any human gene
- 100 transcription factor response element reporters for screening transcription factor activity
- Validated promoter reporters for measuring pathway activation are also available (see page 21)



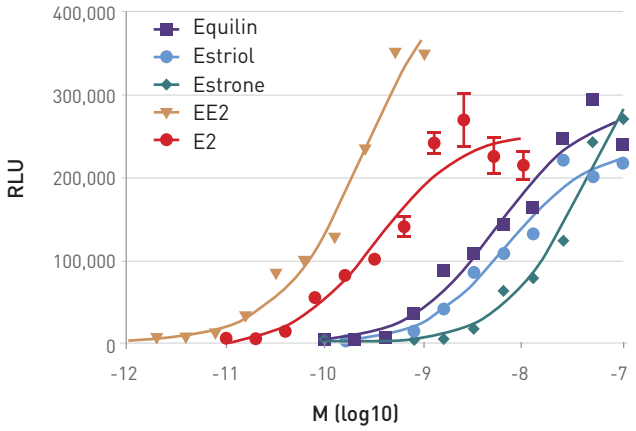


Figure : Dose response of LightSwitch SYT8 Promoter Reporter construct to estrogen compounds. HT1080 cells were co-transfected with a SYT8 Promoter GoClone™ (Product ID S714388) and an ER cDNA expression plasmid, then treated with five different estrogen compounds for 24 hours before the luminescence was measured using the LightSwitch Luciferase Assay Kit.

CUSTOM SERVICES AVAILABLE

- Stable cell line generation
- Custom assay development
- Custom cloning of novel regulatory elements (or TF response elements)

For more complete information, please visit us at www.activemotif.com/lightswitch.

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Enabling Epigenetics Research

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