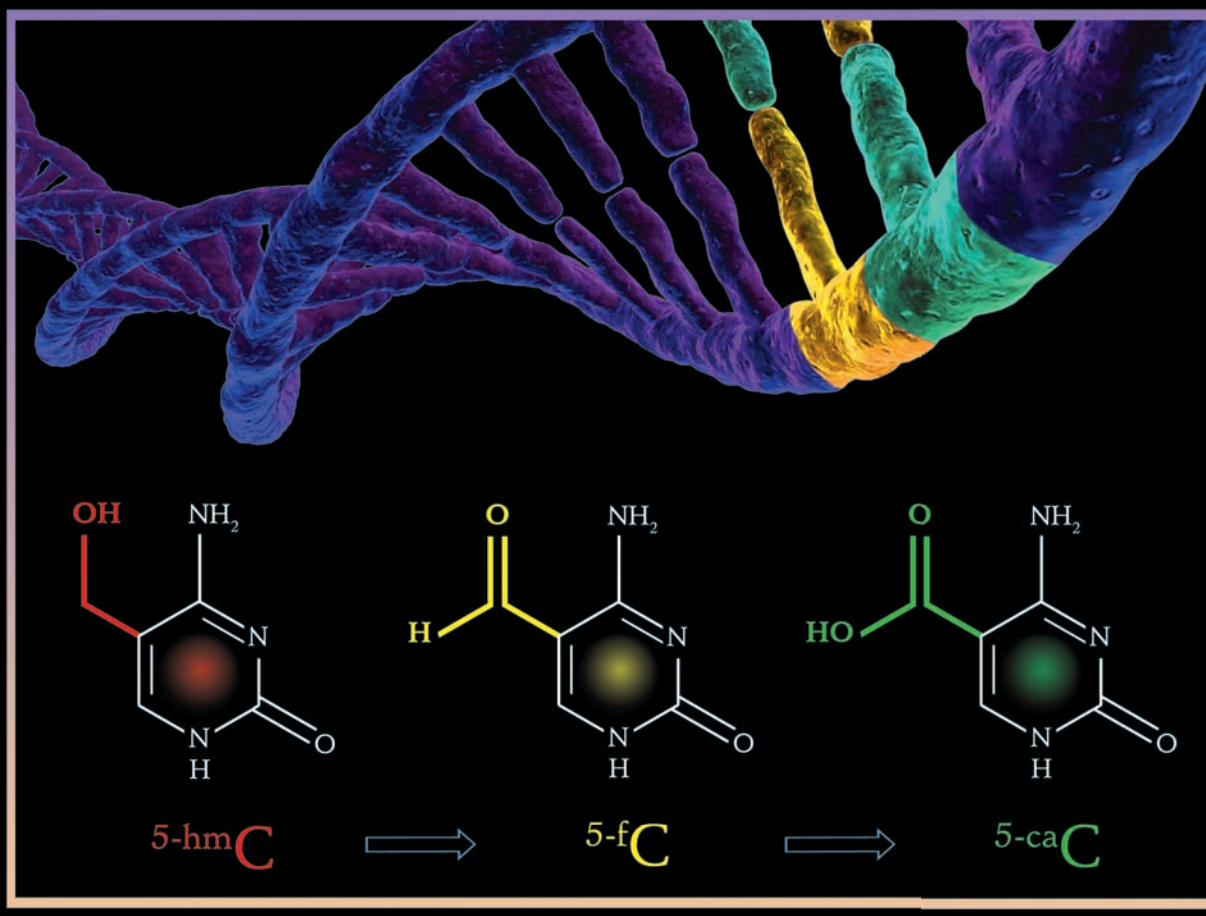
ACTIVE MOTIF®

MOTIFvations

The Newsletter of Active Motif March 2012 Volume 13 Number 1



IN THIS ISSUE

Antibodies, Kits and Services for Epigenetic Research

2 NEW: Antibodies to 5-Carboxylcystosine & 5-Formylcytosine Modifications

2 NEW: DNA Standard to Study 5-Carboxylcytosine

3 Tools to Analyze All Aspects of DNA Methylation

4 Avoid Antibody Bias with Covalent Labeling of 5-Hydroxymethylcytosine

5 NEW: MeDIP-Seq Now Offered by Active Motif Epigenetic Services

6 Polycomb Proteins Help Determine Embryonic Stem Cell Identity

8 HAT & HDAC Assays to Easily Screen for Changes in Histone Acetylation

8 Antibodies & Enzymes for the Study of Histone Acetylation

9 Navigate the Active Motif Website with the Greatest of Ease

10 NEW: Antibodies and Reagents for Super-resolution GSDIM Microscopy

11 NEW: ChIP-IT® Control Kits for Real Time PCR Analysis

11 Expansive Offering of Species-validated Control qPCR Primer Sets for ChIP

NEW: Antibodies to 5-Carboxylcystosine & 5-Formylcytosine Modifications

Active Motif is proud to be at the forefront of research and discovery with the introduction of our new DNA modification antibodies for 5-carboxylcytosine (5-caC) and 5-formylcytosine (5-fC) modifications. While recent studies have focused on the ability of the TET family of cytosine oxygenase enzymes to convert 5-methylcytosine into 5-hydroxymethylcytosine, it now appears that further oxidation of 5-hydroxymethylcytosine results in the formation of 5-formylcytosine and 5-carboxylcytosine.

Even more DNA modifications?

Two recent publications in *Science* by Ito *et al*. and He *et al*., demonstrated that Tet proteins can oxidize 5-hydroxymethylcytosine to generate 5-formylcytosine and 5-carboxylcytosine. Ito *et al*. went on to show that 5-fC and 5-caC are present in mouse embryonic stem cells, while 5-fC could also be identified in major mouse organs. Following the oxidative pathway where 5-methylcytosine is converted to 5-carboxylcytosine (see page 3), it is then believed that 5-caC is excised from genomic DNA by thymine DNA glycosylase (TDG); thereby illustrating a mechanism of DNA demethylation.

Further analysis of 5-fC and 5-caC by Inoue *et al*. in *Cell Research*, revealed that 5-fC and 5-caC appear in the paternal pronucleus after fertilization, concomitant with the disappearance of 5-methylcytosine, and that the levels of 5-fC and 5-caC are gradually diluted out by DNA replication (Figure 1).

Working with the lab of Yi Zhang, Howard Hughes Medical Institute Investigator at the University of North Carolina at Chapel Hill, Active Motif now offers antibodies against these cytosine derivatives. The antibodies have been characterized in dot blot and immunostaining experiments and are available as either whole-rabbit serum or purified IgG. Visit www.activemotif.com/dnamethabs for a complete list of available antibodies.

References
Ito *et al.* (2011) *Science* 333: 1300-1303.
He *et al.* (2011) *Science* 333: 1303-1307.
Inoue *et al.* (2011) *Cell Research* 21: 1670-1676.

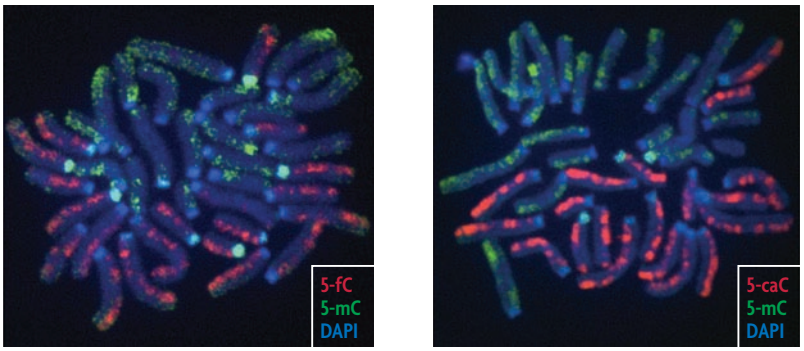


Figure 1: Replication-dependent loss of 5-fC and 5-caC revealed in a 2-cell metaphase embryo.
Shown are representative immunofluorescent images of mitotic chromosome spreads that have been co-stained with Active Motif's 5-Formylcytosine (5-fC) or 5-Carboxylcytosine (5-caC) antibodies (red, Catalog Nos. 61223 and 61225, respectively), a 5-methylcytosine (5-mC) antibody (green) and DAPI (blue) at the two-cell stage of mouse preimplantation development. The 5-fC and 5-caC antibodies were used at a 1:2000 dilution. The images reveal that at the two-cell stage, only one of the two sister chromatids is enriched for 5-fC and 5-caC, consistent with findings that 5-fC and 5-caC levels are diminished by half in blastomeres with each round of DNA replication (Inoue *et al.* (2011)).

NEW: DNA Standard to Study 5-Carboxylcytosine

Control DNA for positive confirmation

To complement the antibodies now available for 5-carboxylcytosine, Active Motif also offers the 5-Carboxylcytosine DNA Standard. This double-stranded DNA sequence contains a total of 12 carboxylcytosine modifications, (8 modifications on the forward strand and 4 modifications on the reverse strand). The 5-Carboxylcytosine DNA Standard contains both modified and unmodified DNA standards with enough material to perform ten dot blots each.

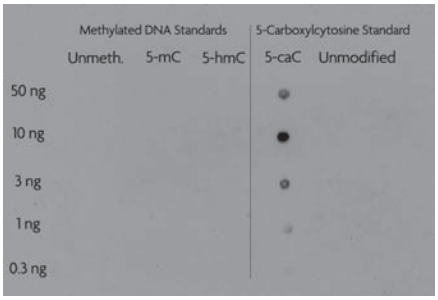


Figure 2: 5-Carboxylcytosine DNA standard.
Varying amounts of dsDNA from the Methylated DNA Standard Kit (Cat No. 55008) or 5-Carboxylcytosine DNA Standard were spotted onto a nylon membrane and probed with Active Motif's 5-Carboxylcytosine antibody (Catalog No. 61225, 1:2000 dilution).

Product	Format	Catalog No.
5-Carboxylcytosine DNA Standard	0.5 µg	55014
5-Carboxylcytosine pAb (Serum)	100 µl	61225
5-Carboxylcytosine pAb (IgG)	100 µg	61229
5-Formylcytosine pAb (Serum)	100 µl	61223
5-Formylcytosine pAb (IgG)	100 µg	61227

Tools to Analyze All Aspects of DNA Methylation

The identification of 5-hydroxymethylcytosine (5-hmC), a derivative of the well-characterized 5-methylcytosine (5-mC) epigenetic mark, established a precedent for the role of Tet enzymes in catalyzing DNA demethylation as a mechanism for epigenetic reprogramming. Recent studies reveal that the pathway of demethylation may involve further oxidation of 5-hmC to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) during cell differentiation and development. To aid researchers in furthering their understanding of how DNA methylation and demethylation work in concert to regulate gene expression, development, and stem cell identity, Active Motif has developed an expansive line of products and technologies to address the most current and cutting edge areas in DNA methylation research. Let our antibodies, assays and enzymes deliver the results you need.

One-stop shop for all your DNA methylation products

To ensure that you have the necessary tools you need at your disposal to succeed with your DNA methylation research, Active Motif offers an expansive portfolio of products to cover the full spectrum of DNA methylation analysis (see Figure 1). Whether your studies require the use of antibodies to distinguish between methyl, hydroxymethyl, formyl, and carboxyl cytosine modifications, Tet enzymes to use for 5-hmC conversion assays, or kits to enrich for methylated or hydroxymethylated DNA, Active Motif has you covered with a wide range of antibodies, kits, and recombinant proteins to address your specific research needs. For complete ordering information or to learn more about all of Active Motif's DNA methylation products, please call or visit us at www.activemotif.com/dnamt.

The Active Motif advantage

- **Convenience** – one-stop shop for all your DNA methylation products
- **Choice** – expansive offering of products including antibodies, kits and recombinant proteins
- **Cutting edge** – products and technologies relevant to the most current research
- **Consistency** – our optimized reagents guarantee consistent and reliable results

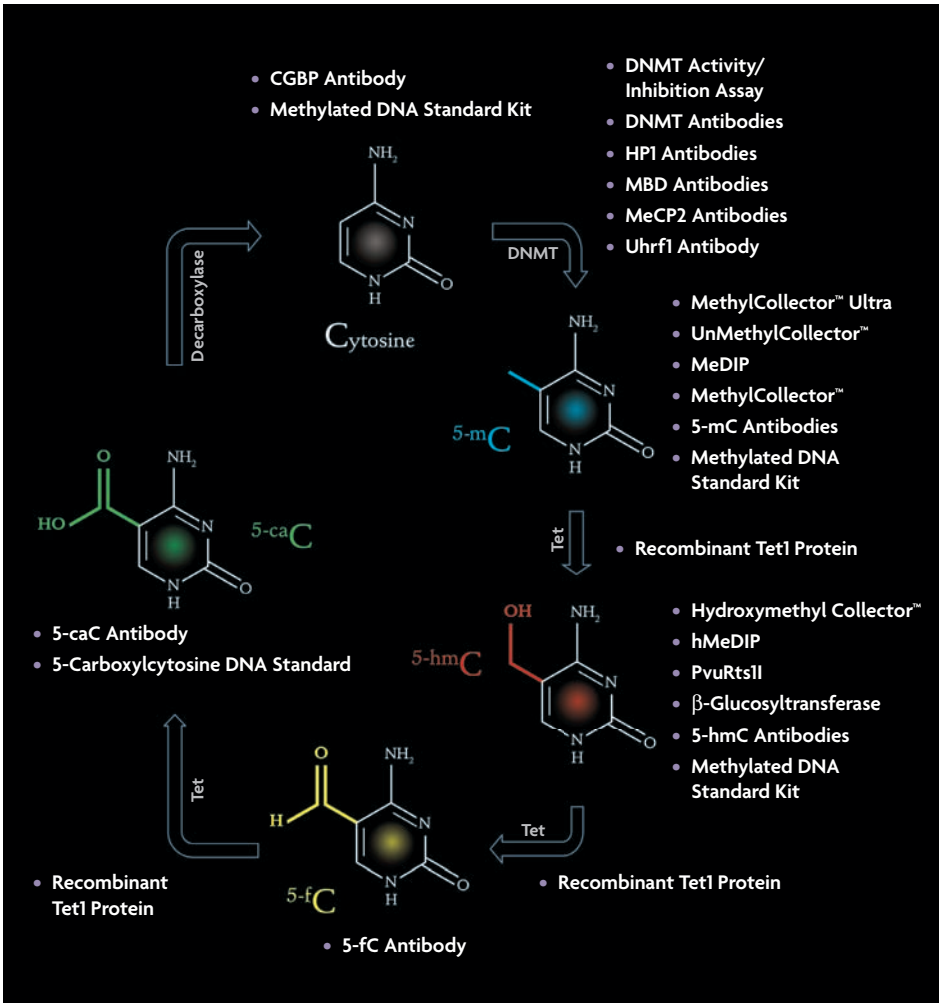


Figure 1: Schematic representation of the oxidation of 5-methylcytosine by Tet enzymes and related products. Tet enzymes catalyze the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC) via oxidation of the methyl group at the 5-position on the cytosine ring. The schematic depicts the demethylation pathway and the Active Motif products associated with the individual steps of the reaction.

Avoid Antibody Bias with Covalent Labeling of 5-Hydroxymethylcytosine

Active Motif's Hydroxymethyl Collector™ Kit is the only commercially available technology to utilize a covalent labeling method to enrich for DNA fragments containing 5-hydroxymethylcytosine (5-hmC). The covalent labeling method avoids sequence and density bias that can be seen with antibody enrichment methods. This method also enables greater sensitivity in the detection of 5-hmC fragments and allows for more complete DNA methylation analysis.

How does it work?

The Hydroxymethyl Collector™ method utilizes the ability of β-glucosyltransferase to modify 5-hydroxymethylcytosine residues by addition of a glucose moiety. When fragmented genomic DNA is incubated in the presence of β-glucosyltransferase and a UDP-Azide-Glucose donor, the enzyme modifies the 5-hmC residue, creating glucosyl-5-hydroxymethylcytosine[1]. Modification of 5-hmC by β-glucosyltransferase is independent of DNA sequence or structural context[2].

The modified 5-hmC DNA is then covalently linked to a biotin conjugate. Magnetic streptavidin beads and the included bar magnet are used to capture the biotinylated 5-hmC DNA fragments. Due to the high affinity between biotin and streptavidin, Hydroxymethyl Collector is capable of capturing DNA fragments with as few as two hydroxymethylcytosine residues per DNA strand. The biotin/streptavidin interaction also allows for high stringency washing conditions to minimize non-specific binding.

Elution buffer cleaves the linker between the biotin and the glucosyl-5-hydroxymethylcytosine, releasing the enriched DNA from the magnetic beads. The eluted DNA is collected and purified with the included purification reagents, leaving you with DNA that is ready for analysis of individual loci by PCR (Figure 2), or whole-genome analysis by microarray or sequencing.

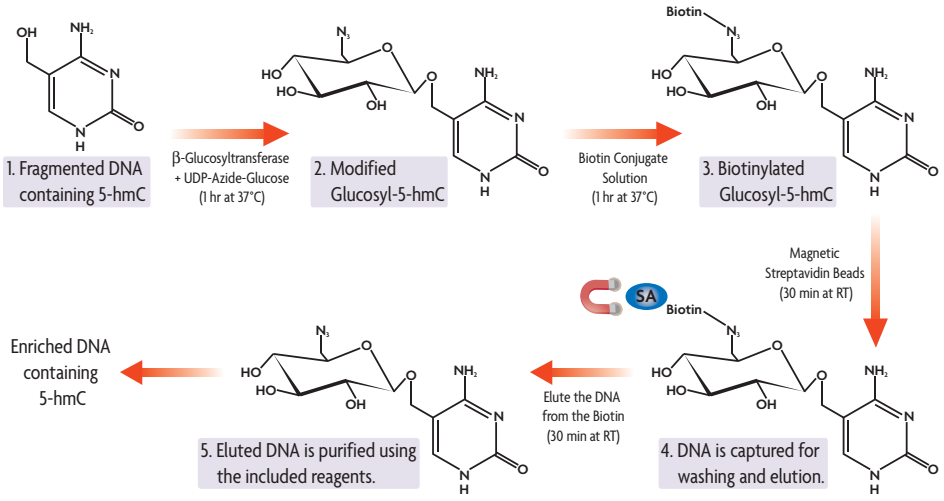


Figure 1: Flow chart of Hydroxymethyl Collector method.
Fragmented DNA (100-500 bp size range) is modified by addition of a glucose moiety. A biotin conjugate is then covalently attached to the glucose enabling capture with magnetic streptavidin beads. Enriched DNA is eluted by cleavage of the biotin linker to release the DNA from the magnetic beads. Eluted DNA is purified before use in downstream applications.

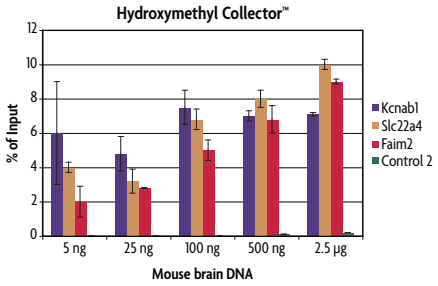


Figure 2: Real-time PCR analysis of the enrichment of the 5-hmC DNA from mouse brain.
Mouse brain DNA was assayed at various quantities in the Hydroxymethyl Collector Kit. Enriched DNA was analyzed by real time PCR across multiple loci. The enriched DNA was quantified and plotted as a percentage of the starting material. Kcnab1, Slc22a4 and Faim2 are methylated targets and Control 2 is unmethylated.

What are the advantages?

- **Covalent labeling** – ensures accurate capture of DNA fragments containing hydroxymethylcytosine
- **Highly sensitive** – technique is sensitive enough to enrich for DNA fragments containing as few as two 5-hmC residues per strand
- **Simple procedure** – can be completed in less than 4 hours
- **Positive control** – 5-hydroxymethylated DNA and real time PCR primers are included to confirm the success of the enrichment reactions

To learn more about all of Active Motif's 5-Hydroxymethylcytosine products, please call or visit us at www.activemotif.com/hmc.

Product	Format	Catalog No.
Hydroxymethyl Collector™	25 rxns	55013

References
1. Song *et al.* (2010) *Nature Biotechnology* 29: 68-72.
2. Szwagierczak *et al.* (2010) *Nucl. Acids Res.*, 38: e181.

NEW: MeDIP-Seq Now Offered by Active Motif Epigenetic Services

The reliable identification of differential DNA methylation is important for researchers interested in biomarker identification, as well as for those trying to understand the basis of disease, drug mechanism of action or environmental influences on epigenetics. To help speed research in these areas, Active Motif now offers MeDIP-Seq as an end-to-end, genome-wide epigenetic service to identify differentially methylated regions.

How does MeDIP-Seq work?

In MeDIP-Seq, a highly specific antibody that recognizes 5-methylcytosine is used to immunoprecipitate sonicated genomic DNA, resulting in the enrichment of genomic regions that are methylated. Because 5-methylcytosine antibody binds only to methylated cytosines in the context of single-stranded DNA, the DNA must be denatured prior to immunoprecipitation. As a result of denaturation the enriched DNA can not be processed for Next-Gen sequencing using the typical sequencing library generation protocols, as these require adaptor ligation to double-stranded DNA. This problem is circumvented by ligating the Next-Gen sequencing adaptors to genomic DNA prior to the immunoprecipitation. Following MeDIP, the enriched regions can be directly amplified with Next-Gen sequencing compatible primers. Unique alignment of the sequence tags across the genome reveals the regions of DNA methylation.

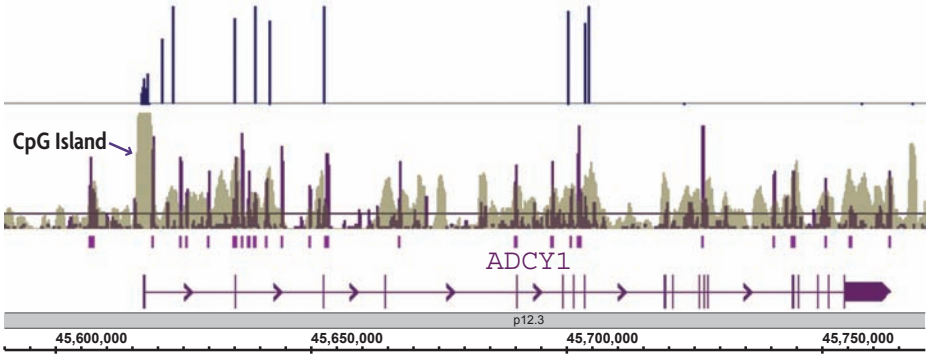


Figure 1: MeDIP-Seq data compared to CpG density and RRBS.
MeDIP-Seq was performed using 2 µg of DNA from human PBMCs. Methylation peaks were mapped across the genome and the image is zoomed in to look at the ADCY1 gene. The middle panel shows good correlation of MeDIP signal (purple bars) with CpG density (overlaid gray peaks) indicating that MeDIP covers most CpG sites across the ADCY1 gene. RRBS data is shown at the top as blue bars. and coverage is limited to only a few regions.

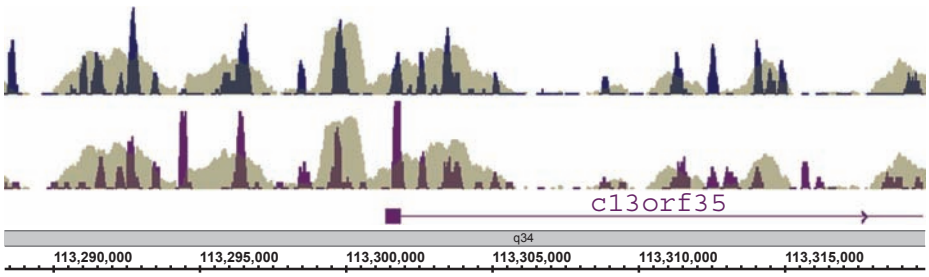


Figure 2: Results from Active Motif's MeDIP-Seq Service correlate well with published MeDIP results.
This image shows a 30 Kb region on chromosome 13. The top panel is data from a published, publicly available data set while the bottom was produced by Active Motif's MeDIP-Seq Service. Overlaid gray peaks show CpG density across the region.

MeDIP-Seq advantages

Several methodologies exist that can detect DNA methylation on a genome-wide scale. Whole genome bisulfite sequencing is the most comprehensive, providing single base resolution across the entire genome. However, it requires sequencing of the entire genome, which is extremely expensive. Reduced Representation Bisulfite Sequencing (RRBS) also yields single base resolution, but it only interrogates 10% of all CpGs and it is more heavily biased toward CpG-rich regions than any other technique. This is not necessarily an advantage as most CpG islands are unmethylated, and because there is growing interest and focus on regions with lower CpG density. MeDIP-Seq coverage is not as biased toward high CpG density and in theory can interrogate all regions across the genome. The expanded genomic coverage of MeDIP-Seq compared to RRBS increases the likelihood of identifying differentially methylated regions in multi-sample studies (Figure 1).

The MeDIP-Seq Service includes

The customer submits purified DNA, frozen tissues or cell pellets, then we:

1) Prepare the sample
2) Perform MeDIP with 5-mC mAb
3) Amplify the enriched DNA sample
4) Perform Next-Gen sequencing
5) Analyze the data

Other Services offered include

- hMeDIP-Seq & ChIP-Seq
- Bisulfite Sequencing
- ChIP Antibody validation, & more

Polycomb Proteins Help Determine Embryonic Stem Cell Identity

Embryonic stem cells (ESCs) are cells derived from the inner cell mass of the developing pre-implantation embryo that gives rise to endoderm, ectoderm and mesoderm. ESCs are unique from all other cells types in that they can differentiate into essentially all cell types in the developing and adult organism, a phenomenon termed pluripotency. They also have the ability to divide into cells that have the same developmental state as the parent (self-renewal). The ability of a cell to remain pluripotent and to self-renew requires the establishment of very complex gene regulation programs. Embryonic stem cells must facilitate the expression of genes required for self-renewal and pluripotency, but at the same time maintain all genes involved in lineage commitment and differentiation in a repressed state.

Unlimited developmental potential

Unlike the cells that are derived from them, embryonic stem cells by their very nature have unlimited developmental potential. Differentiated cells however, like a neuron or muscle cell, have lost the ability (without intervention by the researcher) to be anything other than what they have become. Because ESCs are genetically identical to all of the cells that are derived from them, the primary difference between them is not explained by genetics but rather by epigenetics.

Stem cell identity

The Polycomb proteins are important epigenetic determinants of stem cell identity. They play an important role, serving to help determine whether stem cells remain pluripotent or commit to a more specific cell fate. Originally identified in *Drosophila* as repressors of genes involved in body plan, Polycomb proteins are segregated into one of two protein complexes, Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2; see Table 1). As some Polycomb complex subunits have multiple potential component proteins (*e.g.* there exist four isoforms of EED), various versions of PRC1 and PRC2 complexes have been identified.

PRC1

Protein	Function
BMI-1, MEL18, MBLR, NSPC1	Unknown, required for self-renewal
CBX2/PC1, CBX4/PC2, CBX6, CBX7 & CBX8/PC3	Chromodomain proteins, bind H3K27me3
PHC1, PHC2, PHC3	Unknown
RING1, RING1b/RNF2	Monoubiquitylation of H2A K119

PRC2

Protein	Function
EED (four isoforms)	Stimulation of Lys27 methylation
EZH2, EZH1	H3K27 HMTase
SUZ12	Unknown, required for ESC differentiation
RbAp48	Histone chaperone

Table 1: Polycomb complexes and subunits.

Polycomb response elements

In flies, Polycomb complexes are recruited to specific DNA sequences (termed Polycomb Response Elements or PREs) through the DNA binding protein Polyhomeotic (Ph). Although no mammalian PRE sequences have been identified, there are several potential DNA-binding recruitment proteins, including OCT4, YY1, JARID2, PCL2/MTF2 and AEBP2. Non-coding RNAs, such as HOTAIR, have also been implicated in the recruitment of PRC2.

Once associated with chromatin, the PRC2 subunit EZH2 catalyzes trimethylation of histone H3 at Lys27 (H3K27me3; see Figure 1). H3K27me3 has long been known as a hallmark of regions of repressed chromatin. Trimethylation of Lys27 leads to the recruitment of PRC1 through the binding of H3K27me3 by chromodomain-containing proteins in PRC1. Interaction of PRC1 with non-adjacent regions of chromatin marked by H3 Lys27 trimethylation may contribute to silencing through the establishment

of a domain of specialized chromatin structure that represses gene expression. PRC1 also includes a histone modifying enzyme, Ring1b, which catalyzes the monoubiquitylation of histone H2A at Lys119 (H2AK119ub1). H2AK119ub1 has been found to repress transcript elongation by RNA polymerase II.

The above recruitment and repression model is more complex than described, as many key regulatory genes in stem cells are found in "bivalent" chromatin domains. These bivalent domains, which exhibit hallmarks of both active (H3K4me3) and repressed (H3K27me3) chromatin, are thought to represent a gene poised for either expression or repression. Upon differentiation, bivalent domains are resolved, depending upon the resulting expression state. Genes that are expressed in the target cell type maintain histone H3 Lys4 methylation. Markers of other cell types lose Lys4 methylation but retain the Lys27 methylation and thus go from poised to silent.

In addition to cell type-specific genes and those involved in lineage commitment and differentiation, targets of Polycomb repression in stem cells include tumor suppressors, to prevent cell senescence and potentially promote proliferation. Genes involved in apoptosis are also repressed in a Polycomb-dependant fashion. In terminally differentiated cells, Polycomb proteins participate in the repression of pluripotency and stem cell-specific genes, as well as genes expressed in other cell types.

Summary

It is essential to regulate stem cell self-renewal and orchestrate the differentiation process, and it is clear that Polycomb proteins are a crucial part of this regulation. Cells lacking EED or SUZ12 show aberrant expression of lineage-specific genes which interfere with their ability to differentiate properly. Differentiation is also hampered by the inability to repress pluripotency genes. Mutations in Polycomb proteins are also linked to the establishment of cancer, as aberrant Polycomb function can produce cancer stem cells that exhibit inappropriate differentiation and perpetual self-renewal.

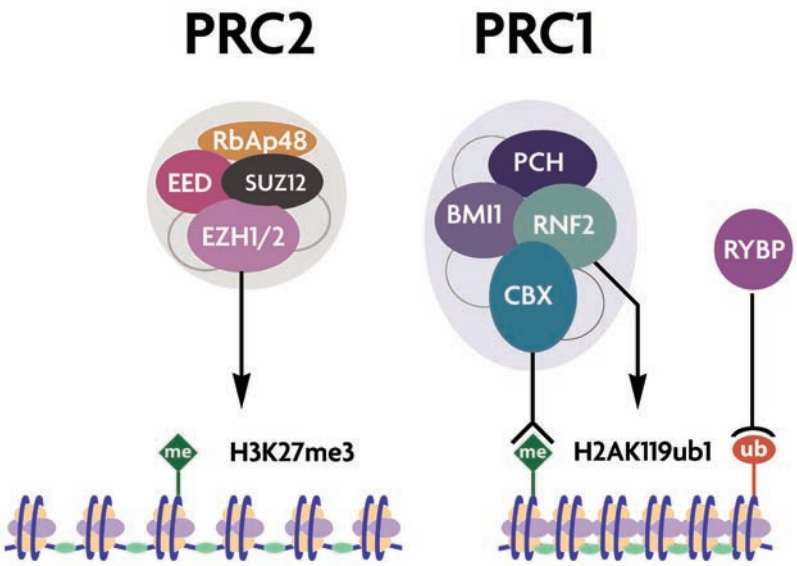


Figure 1: Simplified model of Polycomb Group (PcG)-mediated repression.
PRC2 is recruited to chromatin which allows trimethylation of histone H3 Lys27 by EZH2. PRC1 is then recruited to chromatin through the recognition of H3K27me3 by chromobox (CBX) proteins via the chromodomain. RNF2/RING1 homologs are E3 ubiquitin ligases for H2A, which is monoubiquitylated at Lys119. The RYBP repressor protein recognizes H2A monoubiquitylation, contributing to transcriptional repression. Combined, these activities induce and maintain transcriptional repression. Gray circular outlines depict other PRC subunits and associated proteins.

STEM CELL ANTIBODIES

Active Motif has a large and expanding list of antibodies to proteins important in stem cell biology. Visit www.activemotif.com/stemcellabs for the complete list.

Product	Format	Catalog No.
BMI-1 antibody (mAb)	100 µg	39993
CBX8 antibody (pAb)	100 µl	61237
EED antibody (mAb)	100 µg	61203
EZH2 antibody (mAb)	100 µg	39875
EZH2 phospho Thr345 antibody (pAb)	100 µl	61241
Histone H3 trimethyl Lys27 antibody (mAb)	100 µg	61017
PCL2 antibody (mAb)	100 µg	61153
Phc1 antibody (mAb)	100 µg	39723
Phc2 antibody (mAb)	100 µg	39661
Ring1B antibody (mAb)	100 µg	39663
Suz12 antibody (mAb)	100 µg	39877
YY1 antibody (pAb)	100 µl	39071

Better Solutions for the Analysis of Histone Acetylation & Deacetylation

Histone acetylation and its antagonistic counterpart, histone deacetylation, play a major role in chromatin structure and gene expression. Acetylation of lysine residues on histone proteins leads to a less compact chromatin configuration that is associated with gene expression. Acetylation of histones H3K9, H3K18, H4K12 and H4K16 are associated with diseased states and could be potential targets in therapeutic studies. To better understand the role of histone acetylation and deacetylation on chromatin structure and gene expression, Active Motif offers assays, antibodies and enzymes for the analysis of these modifications.

HAT & HDAC Assays to Easily Screen for Changes in Histone Acetylation

How does the HAT Assay work?

The HAT Assay Kit is a quick and sensitive method to determine the activity of your own source of purified histone acetyltransferase (HAT), or to screen for potential inhibitors of HAT activity. The fluorescent assay includes both histone H3 and H4 N-terminal histone tail substrates. HATs will catalyze the transfer of acetyl groups from the provided acetyl-CoA to generate an acetylated peptide and CoA-SH. After stopping the reaction with stop solution, a developer is added that reacts with the free sulfhydryl groups on CoA-SH to give a fluorescent signal (Figure 1). A standard curve can be generated in order to relate the fluorescence of your HAT to pmol/min/µg specific activity.

Histone Deacetylase (HDAC) Assay Kit

For researchers interested in studying histone deacetylation, Active Motif also offers both a colorimetric and fluorescent HDAC Assay Kit. The kit contains an acetylated peptide substrate that can be deacetylated by Class I, IIB and IV HDAC enzymes. A deacetylated assay standard is provided to enable calculation of HDAC activity in pmol/min/mg.

To download a copy of our Histone Analysis Products brochure, go to www.activemotif.com/info.

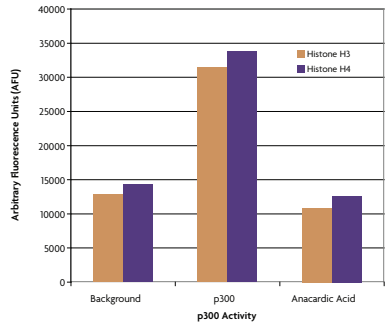


Figure 1: HAT inhibitor effects on p300 activity. Fifty nanograms of the acetyltransferase p300 were assayed per well with 50 µM acetyl-CoA and 50 µM histone H3 or H4 peptide substrates in the absence or presence of 15 µM anacardic acid, a known HAT inhibitor. The background signal indicates the level of auto-acetylation present from the p300 acetyltransferase.

Product	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Antibodies & Enzymes for the Study of Histone Acetylation

Need an active HAT or HDAC?

Whether you are looking for a control enzyme to compare alongside your own source of HAT or HDAC, or if you want an enzyme for inhibitor screening, Active Motif offers active recombinant proteins for the study of histone acetylation and histone deacetylation.

- **p300**
- **GCN5**
- **HDAC 1-9**
- **SIRT1 & SIRT6**

Histone acetyl antibodies

Visit our website to see the complete list of available histone antibodies.

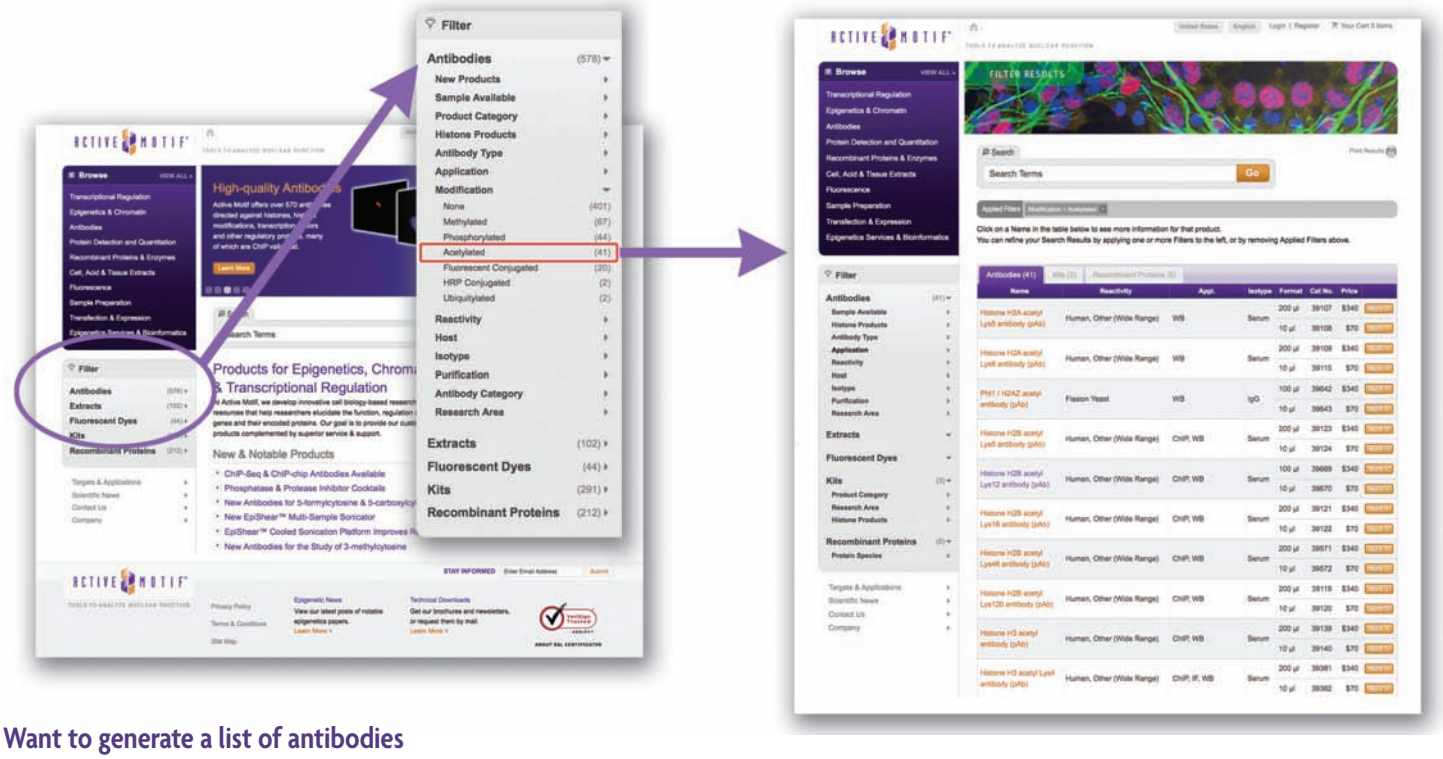
- **Large portfolio** includes more than 40 antibodies specific to histone acetylation modifications
- **Versatile target selection** contains antibodies for H2A, H2B, H3 & H4 acetylation targets
- **Validated applications** include ChIP, ChIP-seq, ChIP-chip, IF and WB
- **Sample size antibodies available**

Try our search filters to streamline your search results

To browse the complete list of available antibodies and enzymes, please visit our website at www.activemotif.com. Use our search filters on the left-hand side of our web page to narrow your results based on, for example, antibody "Modification" or recombinant protein "Protein Category". (See page 9 for more information on using the search filters on our website).

Navigate the Active Motif Website with the Greatest of Ease

Active Motif has recently added "Faceted Search" to its website. This enables you to perform very specific searches using the Filters located in the left-hand column of every page. Multiple Filters can be applied at the same time and they can also be combined with Search Terms, so you can find the products you want faster. See below for examples.



Want to generate a list of antibodies for a specific type of modification?

To display a list of high-quality antibodies specific for acetylated proteins, the first step is to click on "Antibodies" under Filters in the left column. This expands "Antibodies" so that all subcategories that can be applied as Filters are displayed. (The numbers shown in parentheses tell you how many products are in each category; these will update as you apply Filters.) One of the Filters is "Modification". Clicking on this expands "Modification" so that it displays all types of modification-specific antibodies available. Selecting "Acetylated" will take you to a Search Results page in which the Antibodies tab shows all of our antibodies against acetylated targets. (As we also offer Kits and Recombinant Proteins that meet the search criteria for "Acetylated", tabs for these are also displayed above the results chart.)

Search Results can be further narrowed by applying additional Filters. For example, under "Application" you could click on "Chromatin Immunoprecipitation", which would then update the results so they display ChIP-validated antibodies against acetylated proteins. (Note that the tabs for Kits and Recombinant Proteins disappear, as no products of those types meet both search criteria.)

Combine Search Terms with Filters

You can also apply Keyword searches in combination with one or more Filters. In our current example, if you type "H3" in the Search Terms box then click "Go", the Search Results will become even more specific. Now, only ChIP-validated Histone H3 antibodies that are against acetylated proteins are found. Filters can continue to be applied until the found results cannot be narrowed any further.

Filters will disappear if they will not change the Results. For example, if you have generated a Search Result in which all antibodies are Polyclonal, "Antibody Type" will longer be displayed as a Filter as applying it won't change the results.

Removing Filters is also useful

Finally, Applied Filters can be removed in any order by clicking the "x" next to its name in the gray bar above the results. To demonstrate, click on the "x" next to "Chromatin Immunoprecipitation". The Search Results now display products under the Antibodies, Kits and Recombinant Proteins tabs that meet the "H3" and "Acetylated" search criteria.

Find what you're looking for faster

Faceted Search enables you to quickly find the specific products you're looking for. Try it today at www.activemotif.com.

NEW: Antibodies and Reagents for Super-resolution GSDIM Microscopy

Until recently, fluorescence microscopy has been limited by the level of resolution that can be achieved in imaging experiments. The current diffraction limit is approximately 200 nm and does not allow the evaluation of the nano-architecture of the cell or the detection of cellular events in molecular detail. Ground State Depletion with Individual Molecule return (GSDIM) is one of the super-resolution techniques that has been developed to overcome these limitations. GSDIM provides the ability to image structures as small as 20 nm. These improvements in microscopy enable scientists to decipher the nanostructure of the cell in details that previously could not be resolved (Figure 1).

In regular fluorescence microscopy, diffraction limits image resolution to roughly half the wavelength of the emitted light. Individual fluorescent dye molecules often cannot be optically separated as they are too close. In Figure 2A, the dyes in the excitation spot will overlap and appear to be one signal.

Super-resolution by localization

In GSDIM, the majority of dye molecules within the excitation spot are transformed into a dark state; only some single dye-molecules, which are well separated from one another, are able to emit fluorescence (Figure 2B). After these molecules are imaged with a camera, a mathematical algorithm determines the locations of the next set of individual dye molecules that will be allowed to fluoresce, and another image is created (Figure 2C). This is repeated thousands of times. By overlaying all of the images, a high-resolution image of the sample is constructed. With GSDIM, a resolution of 20 nm is possible.

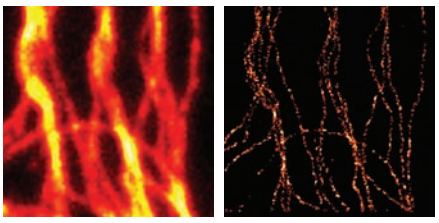


Figure 1: Active Motif's fluorescent secondary antibodies in GSDIM microscopy.
HeLa cells were stained with alpha Tubulin mouse mAb (Clone 5-B-1-2) and Chromeo™ 505 Goat anti-mouse IgG (Catalog No. 15030). The widefield image (left) and the GSDIM image (right) are provided courtesy of Leica Microsystems, Germany.

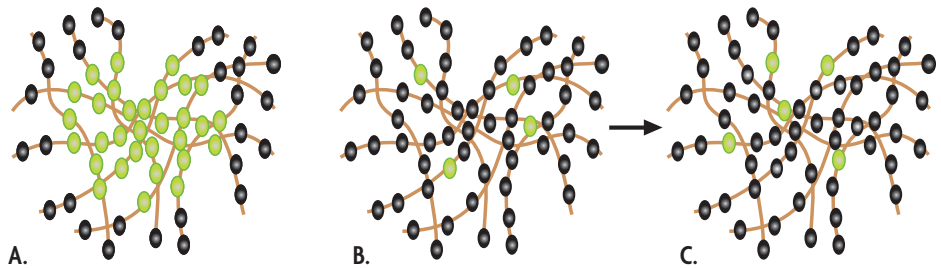


Figure 2: The principle of GSDIM microscopy.
Illustration of the exposed area of a sample in classical widefield microscopy (A) or in GSDIM microscopy (B + C) in which only a small number of spatially separated, single dye molecules are allowed to emit fluorescence at the same time. The spheres represent individual dye molecules in fluorescent (green) or "off" mode (black).

In contrast to other stochastic methods, GSDIM has the advantage that regular, high-quality fluorescent dyes can be used, which allows researchers to work with their regular staining protocols. Accordingly, Active Motif's IF-validated primary antibodies, high-quality Chromeo™-, ATTO- and Rhodamine 6G-conjugated secondary antibodies and fluorescent Chromeo Dyes will help researchers take advantage of the new, powerful GSDIM technology, ensuring the highest quality images possible.

Advantages of Active Motif's dyes and secondary antibodies for use in super-resolution microscopy

- Brilliant
- Stable
- Low background
- Optimized conjugation protocols
- Recommended by Leica for use with its SR GSD microscope

For more information on GSDIM and our fluorescent dyes and secondaries, please visit us at www.activemotif.com/gsdim

Product	Format	Catalog No.
Chromeo™ 488 Goat anti-Mouse IgG	1 mg	15031
Chromeo™ 488 Goat anti-Rabbit IgG	1 mg	15041
Chromeo™ 505 Goat anti-Mouse IgG	1 mg	15030
Chromeo™ 505 Goat anti-Rabbit IgG	1 mg	15040
Chromeo™ 546 Goat anti-Mouse IgG	1 mg	15033
Chromeo™ 546 Goat anti-Rabbit IgG	1 mg	15043
ATTO 532 (GSD) Goat anti-Mouse IgG	250 µl	15070
ATTO 532 (GSD) Goat anti-Rabbit IgG	250 µl	15072
Rhodamine 6G (GSD) Goat anti-Mouse IgG	250 µl	15074
Rhodamine 6G (GSD) Goat anti-Rabbit IgG	250 µl	15076

NEW: ChIP-IT® Control Kits for Real Time PCR Analysis

Optimizing chromatin immunoprecipitation (ChIP) experiments to confirm the quality of your chromatin preparation or validate your antibody for use in ChIP can be difficult without the right controls. To make it easier to determine if your ChIP reactions are working, Active Motif offers a variety of ChIP kits and accessories including control chromatin, ChIP-validated antibodies, control qPCR primer sets and our new species-specific control kits for use in real time PCR analysis. To see a full listing of available ChIP kits and accessories, please visit www.activemotif.com/chip.

ChIP-IT® Control qPCR Kits

In an effort to provide more complete chromatin analysis, Active Motif is launching our new ChIP-IT® Control qPCR Kits for human, mouse and rat. Control kits are ideal for ChIP antibody validation when run in parallel with your antibody of interest. The control kit will confirm the chromatin preparation and IP procedure worked properly and allow you to assess your test antibody.

One of the advantages of using the ChIP-IT Control qPCR Kits is that each kit contains both positive and negative control PCR primer sets. This enables a direct comparison of gene targets for the same antibody and eliminates the variation that can be observed when trying to analyze ChIP results against the negative control IgG, as each antibody has a different binding affinity. All of our control qPCR primer sets have been rigorously tested by our Epigenetic Services division and validated to work for you.

What is included in the Control Kits?

- **RNA pol II antibody** – positive control antibody to verify the ChIP reaction was successful
- **Bridging antibody** – enhances the binding affinity of mouse monoclonal antibody to protein G beads and is recommended for use with all mouse monoclonal antibodies, including the RNA pol II control
- **Negative control antibody** – mouse IgG antibody that can be used to evaluate non-specific binding
- **Positive control qPCR primers** – species-specific control primer set that amplifies the RNA pol II ChIP
- **Negative control qPCR primers** – species-specific control primer set not amplified in the RNA pol II ChIP

Save 30% on new Control Kits

To help you get your ChIP experiments started with suitable controls, for a limited time Active Motif is offering a **30% discount** on all of our new ChIP-IT Control qPCR Kits for human, mouse and rat when you purchase one with a ChIP-IT® Express or ChIP-IT® Express Enzymatic Kit. Use the control kits in combination with either of our ChIP-IT Express Kits, which provide a detailed protocol and optimized reagents for successful chromatin preparation and immunoprecipitation. Simply cite the promotion code **"MV13-1"** when you order to receive your discount.

Product	Format	Catalog No.
ChIP-IT® Control qPCR Kit – Human	5 rxns	53026
ChIP-IT® Control qPCR Kit – Mouse	5 rxns	53027
ChIP-IT® Control qPCR Kit – Rat	5 rxns	53028
ChIP-IT® Express	25 rxns	53008
ChIP-IT® Express Enzymatic	25 rxns	53009

Expansive Offering of Species-validated Control qPCR Primer Sets for ChIP

Need ChIP controls for your species?

Active Motif offers human, mouse, rat, Zebrafish, *Drosophila* and yeast qPCR primer sets for use as positive and negative controls for many of the more common ChIP targets, including histone modifications, transcription factors and methylated DNA (Table 1). To see the full list of over 35 available primer sets and their validated targets, visit our website at www.activemotif.com/chipprimers.

Product	Use as a Control for:	Catalog No.
Human Positive Control Primer Set ACTB-2	Many activating histone marks	71005
Human Positive Control Primer Set MYT1	H3K27me3 & EZH2	71007
Human Positive Control Primer Set ZC3H13	5-mC (MeDIP)	71009
Human Negative Control Primer Set 1	Most TFs & Histone mods, Pol II & 5-mC	71001
Mouse Positive Control Primer Set Hoxc10	H3K27me3 & EZH2	71019
Mouse Negative Control Primer Set 2	Most TFs & Histone mods, Pol II & 5-mC	71012
Drosophila Positive Control Primer Set Act5C	RNA Pol II phospho Ser2	71030
Drosophila Negative Control Primer Set 1	Most TFs & Histone mods, & Pol II	71028

Table 1: A few examples of the many species-specific ChIP Control qPCR Primer Sets offered by Active Motif.