MOTIF VATIONS

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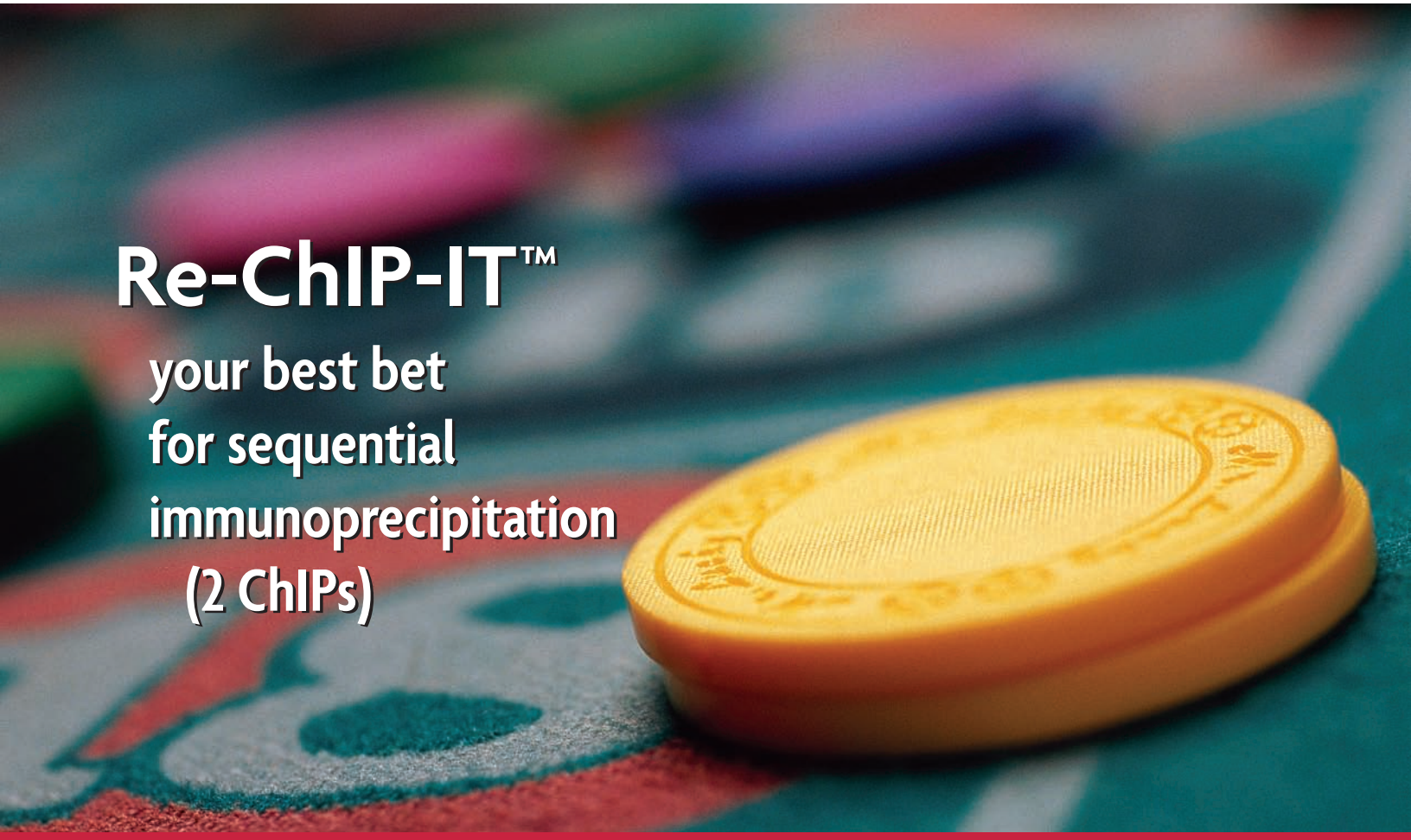
ACTIVE MOTIF®

Tools to Analyze Nuclear Function



Re-ChIP-IT™

your best bet for sequential immunoprecipitation (2 ChIPs)

IN THIS ISSUE

Lower Background Co-IP of Both Nuclear & Whole-cell Complexes

The Universal Magnetic Co-IP Kit includes protein G-coated magnetic beads that simplify the IP and wash steps while providing lower background, which improves your Co-IP results. The kit also includes reagents to prepare both nuclear and whole-cell extracts, so you can study intact protein complexes whether they were originally bound to DNA or in the cytoplasm.

Protein/protein interactions are often studied using co-immunoprecipitation (Co-IP), in which a single antibody is used to precipitate an entire protein complex. Additional antibodies are then used in Western blot to identify/verify other complex members (Figure 1).

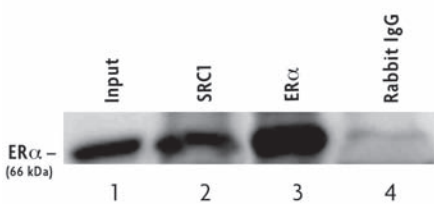


Figure 1: Nuclear Co-IP of SRC-1 and ERα.
The Universal Magnetic Co-IP Kit was used to make nuclear extract from MCF-7 cells induced 1 hour with 10 nM Estradiol. IP was performed on 300 µg samples using 2 µg of SRC-1 pAb, ERα pAb and rabbit IgG (as a negative control). Western blot was then performed using the ERα pAb on 10 µg Input Extract (Lane 1), SRC-1 IP (Lane 2), ERα IP (Lane 3) and the rabbit IgG IP (Lane 4).

Co-IP cytoplasmic AND nuclear complexes

Co-IP is often used to study cytoplasmic protein complexes. But, traditional methods are not optimal for studying DNA-binding proteins because nuclear complexes are very fragile, causing them to be disrupted during extraction. For this reason, in addition to containing components for preparing whole-cell extracts, the Universal Magnetic Co-IP Kit provides nuclear extraction reagents that have been optimized to preserve nuclear protein complexes. The kit's Enzymatic Shearing Cocktail uses DNA digestion to gently release the nuclear protein complexes from the DNA, so they are intact and ready for Co-IP.

Simpler procedure, lower background

The Universal Magnetic Co-IP Kit utilizes protein G-coated magnetic beads, which simplify Co-IP by enabling the IP and wash steps to be performed in seconds, rather than having to use centrifugation. Because these beads have very low non-specific binding, background is reduced even while using the kit's low-salt Co-IP/Wash Buffer, which is designed to help maintain weaker complexes.

Universal Magnetic Co-IP Kit advantages

- Magnetic beads simplify procedure and reduce background
- Optimized extraction method maintains nuclear protein complexes
- Preserve protein modifications

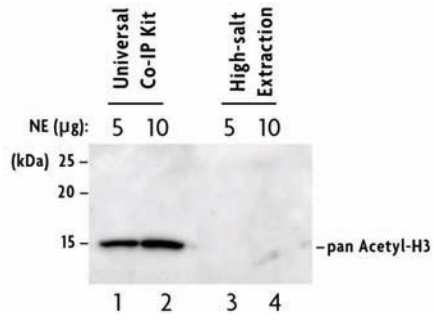


Figure 2: Detection of acetylated histone H3.
HeLa nuclear extracts were made using the Universal Magnetic Co-IP Kit and a traditional high-salt extraction protocol, each supplemented with 1 µM trichostatin A, a deacetylase inhibitor. Five and ten µg samples of each extract were used in Western blot with Histone H3 acetyl rabbit pAb (Cat. No. 39139). Protein was detected only in samples made using the kit's gentle nuclear extraction procedure.

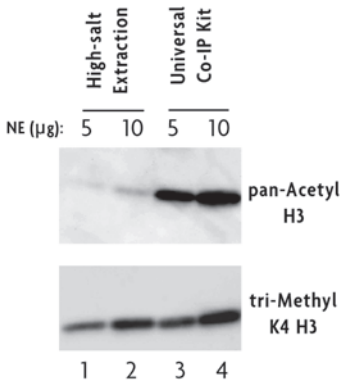


Figure 3: Preservation of acetylation and methylation.
Nuclear extracts were made from HeLa cells treated with 500 nM trichostatin A for 20 hours using either the Universal Magnetic Co-IP Kit (with its deacetylase inhibitor) or traditional high-salt extraction. Five and ten µg samples of these extracts were used in Western blot with Histone H3 acetyl pAb (Cat. No. 39139) and Histone H3 trimethyl Lys4 pAb (Cat. No. 39159). The acetylated protein was detected only in the sample made using the kit. Methylation was slightly better maintained in the sample made using the kit.

Complete kit for better results

The Universal Magnetic Co-IP Kit has both nuclear and whole-cell extraction reagents, so you can perform IP on all types of protein complexes. The kit also includes protein G-coated magnetic beads, a unique Co-IP/Wash Buffer as well as phosphatase, protease and deacetylase inhibitors that preserve the integrity of the proteins and protein modifications (Figures 2 & 3). Finally, the kit includes a strong bar magnet, so you can take advantage of the improved wash and IP steps enabled by the magnetic beads. This makes the Universal Magnetic Co-IP Kit a simple, flexible and complete solution for getting more from your Co-IP. To find out more, please give us a call or visit us on the web at www.activemotif.com.

Product	Format	Catalog No.
Universal Magnetic Co-IP Kit	25 rxns	54002

NEW: Identify Protein Co-localization *In Vivo* Using Sequential Chromatin IP

Performing sequential chromatin IP (also called Re-ChIP) was technically challenging and difficult, until now. Active Motif's new Re-ChIP-IT™ Kit makes it easy to perform sequential ChIP, so you can localize two different proteins or histone modifications to the same genomic locus.

Extend the utility of ChIP

When performing ChIP experiments, it is often useful to prove that two different proteins or histone modifications are present at the same site in the genome. Or, you may want to determine if a protein coincides with a specific histone modification at the same regulatory element. Re-ChIP is a relatively new technique in which sequential chromatin immunoprecipitations are performed using two different antibodies, enabling you to assay for the simultaneous presence of two proteins or distinct histone modifications at the same genomic region of interest (Figure 1).

How does Re-ChIP-IT work?

The new Re-ChIP-IT Kit takes advantage of the same advances introduced in Active Motif's popular ChIP-IT Express Kit. Each method uses protein G-coated magnetic beads that have less background than standard agarose beads, so pre-clearing and blocking steps are not needed. Magnetic pull-down occurs in just seconds, and the low background has eliminated the need for DNA purification. With Re-ChIP-IT, the 1st ChIP is performed as in regular ChIP. Chromatin that has been immunoprecipitated is removed from the magnetic beads with a special buffer that prevents the first antibody from participating in the 2nd IP reaction. After desalting, a 2nd ChIP is performed using an antibody different from that used in the first ChIP. The cross-links of these sequentially immunoprecipitated protein-DNA complexes are then reversed and the DNA is analyzed by PCR, similar to conventional ChIP samples (Figure 2).

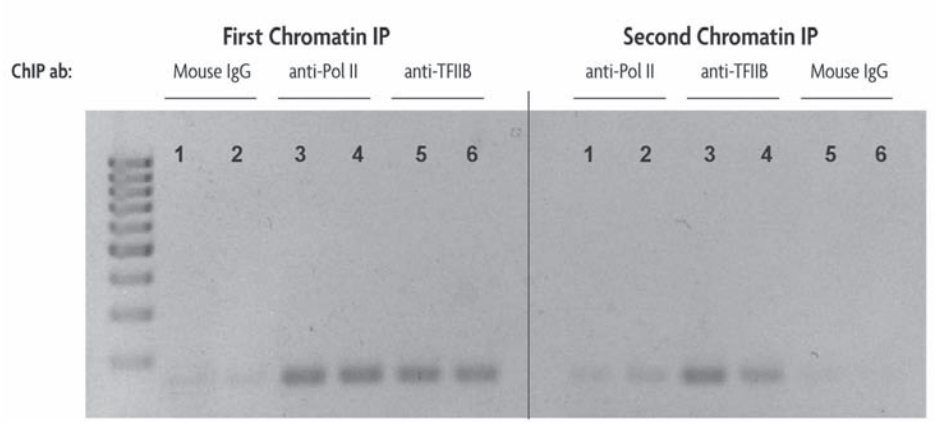


Figure 1: Sequential chromatin immunoprecipitation using Re-ChIP-IT.
The lane numbers are the same in each panel to indicate that the DNA is from the same chromatin sample. The left panel shows the results of PCR performed on an aliquot of DNA removed from the experiment after the first ChIP step; the right panel represents PCR results on DNA from chromatin samples after both ChIP steps. For example, chromatin samples subjected to first ChIP using Mouse IgG as a negative control (lanes 1 and 2 in the left panel) were then subjected to a second ChIP with an RNA Pol II antibody (lanes 1 and 2 in the right panel). Chromatin samples in which Mouse IgG was used as either the first antibody (lanes 1 and 2) or second antibody (lanes 5 and 6) show little amplification of GAPDH DNA in either the left (first ChIP) or right panel (first and second ChIP). Chromatin samples in which the first antibody used was anti-RNA Pol II and the second antibody was anti-TFIIB (lanes 3 and 4) show good amplification of GAPDH DNA after the second ChIP (right panel) indicating co-localization of RNA Pol II and TFIIB at the same region of the GAPDH promoter.

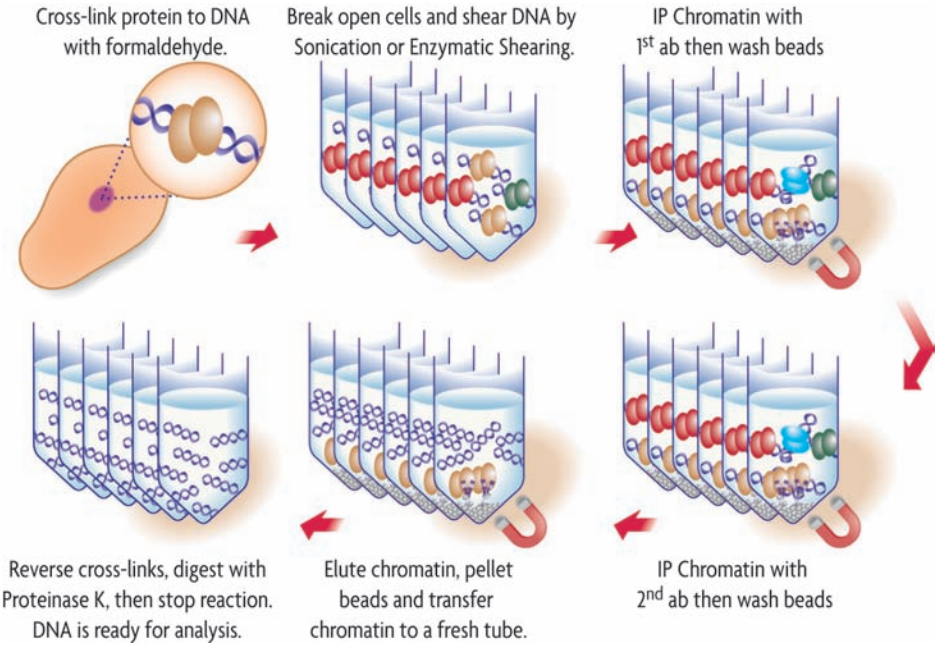


Figure 2: Schematic representation of the Re-ChIP-IT procedure.

Product	Format	Catalog No.
Re-ChIP-IT™	25 rxns	53016

NEW: Site- and Degree-specific Methylated Lysine Histone H3 & H4 Proteins

Active Motif's Recombinant Histones are available with site-specific mono-, di- and trimethylated lysines, so you can investigate the influence of specific lysine methylation on the binding of associated chromatin proteins and nucleosome remodeling. In addition to the methylated histone H3 Lys4, 9 & 27 proteins released previously, we are now introducing methylated histone H3 Lys79 & methylated histone H4 Lys20 proteins.

Histones & chromatin structure

Methyl-lysine residues in nucleosomal histones are thought to mediate interactions with the protein complexes involved in regulating transcription, replication and DNA repair. To better investigate how methylation patterns impact regulatory processes, assays will require histones with specific methylation states. Active Motif recently introduced methylated histone H3 (Lys4, 9 & 27) proteins, and we are now adding methylated histone H4 Lys20 and H3 Lys79 proteins to our line-up. These recombinant histones make it possible to investigate the function of specific methylation states on chromatin biology.

Methylated histone H3 & histone H4

Active Motif is introducing recombinant histone H3 proteins that are mono-, di- and trimethylated at lysine 79 and recombinant histone H4 proteins mono-, di- and trimethylated at lysine 20.

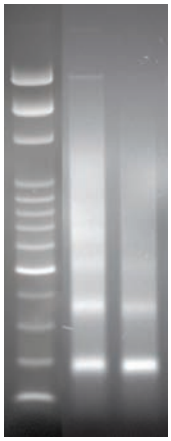


Figure 1: Ordered spacing of nucleosomes after enzymatic digestion of assembled chromatin.
Recombinant Histone H3 dimethyl Lys9 (4.5 µg) was used to generate chromatin *in vitro* using the Chromatin Assembly Kit (Cat. No. 53500). One µg of assembled chromatin was digested for 2 minutes (Lane 2) and 4 minutes (Lane 3). Lane 1 is 100 bp marker.

How is the methylation state made?

Using a patented approach, an analog of methyl lysine has been installed in the histone via a chemical alkylation reaction. With this specific chemical treatment, the site and degree of methylation can be controlled. Each methylation reaction is over 99% complete, and is verified by orthogonal acceleration ESI-TOF mass spectrometry. The recombinant histones are also analyzed by dot blot or immunoblot to confirm identity (Figure 2). The installed methylation state very closely mimics natural methylation, so these recombinant histones are perfect for any functional assay.

Order today!

Visit our website for more info or to try any of these recombinant histones as a standard for your assays or as a building block for specific *in vitro* chromatin assays (Figure 1).

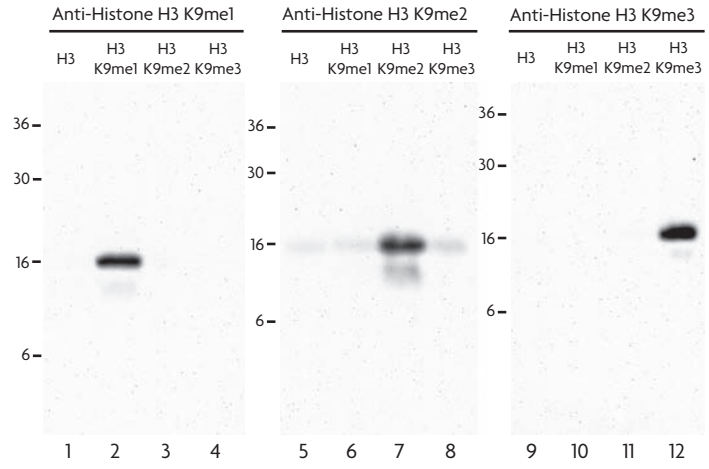


Figure 2: Western blot analysis of mono-, di- and trimethylated Recombinant Histone H3.
1 µg Recombinant Histone H3 (Cat. No. 31207) (lanes 1, 5 & 9), 1 µg Recombinant Histone H3 monomethyl Lys 9 (Cat. No. 31211) (lanes 2, 6 & 10), 1 µg Recombinant Histone H3 dimethyl Lys9 (Cat. No. 31212) (lanes 3, 7 & 11) and 1 µg Recombinant Histone H3 trimethyl Lys9 (Cat. No. 31213) probed with Histone H3 monomethyl Lys9 pAb (Cat. No. 39249) (lanes 1-4), Histone H3 dimethyl Lys9 pAb (Cat. No. 39239) (lanes 5-8) and Histone H3 trimethyl Lys9 pAb (Cat. No. 39161) (lanes 9-12).

Product	Format	Catalog No.
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Please visit www.activemotif.com/recomhistones to see our entire line of Recombinant Histones.

NEW: Sensitive ELISAs to Quantitate Histone Methylation Levels

Active Motif has applied our expertise in making histone-modification antibodies to produce optimal antibody pairs for the detection of specific histone modifications in a sandwich ELISA format. This makes it easy to quickly screen acid extracts for changes in levels of methylation of histone H3 at lysine 4. The kits include our site- and degree-specific methylated recombinant histones for use as a quantitative reference.

Histone H3 Methyl Lys4

Methylation of Lys4 on histone H3 has been correlated with active gene transcription in eukaryotes, making the Lys4 methylation state an interesting marker of transcriptional activity. Active Motif's new Histone H3 methylated Lys4 ELISAs are now available for screening the levels of di- or trimethyl Lys4 in acid extract samples. With the simple format of our ELISA kit, it is easy to assess and quantitate levels of methylated histone H3 Lys4 in acid extracts.

Histone ELISA advantages

- **Sensitive** – works with 100-500 ng of acid extract
- **Specific** – C-terminal histone H3 capture and site- & degree-specific methylation detection
- **Efficient** – no time-consuming immunoblotting
- **Convenient** – strip-well format so you use only what you need

Specific results fast!

Screening extracts by standard immunoblotting methods is time consuming. Our new Histone Modification ELISAs enable you to efficiently screen up to 96 wells at once, with little hands-on time, and to get colorimetric results in just hours. With Active Motif's high-quality modification-specific histone antibodies (see Page 6), accurate results with extremely low background are guaranteed (Figures 1 & 2). Recombinant Histone H3 di- or trimethyl Lys 4 protein (see Page 4) is included in the ELISA kits, so you can compare sample results with known protein amounts for quantitative data on methylation levels.

What's in the ELISA kits?

The Histone ELISA kits come complete with everything you need to quantify changes in the levels of methylation of histone H3 at Lys4:

- A 96 strip-well plate coated with C-terminal histone H3 polyclonal antibody for capture of histone H3
- Biotinylated histone H3 di- or trimethyl Lys4 antibody for specific detection of di- or trimethylated H3
- Recombinant Histone H3 di- or trimethyl Lys4 protein for producing standard curves
- Streptavidin-HRP and all buffers & developing solutions required for colorimetric readout

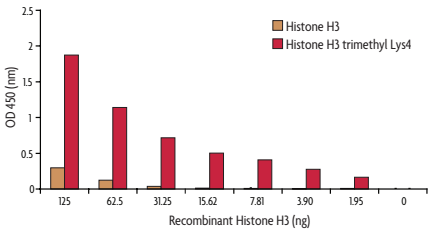


Figure 1: Histone H3 trimethyl Lys4 detection.
1.95 to 125 ng of Recombinant Histone H3 and Recombinant Histone H3 trimethyl Lys4 were assayed using the Histone H3 trimethyl Lys4 ELISA.

Quantitate methylation levels

By including our unique Recombinant Histone H3 proteins with site- and degree-specific methylation states, methylation levels in sample acid extracts can easily be quantitated from a reference standard curve. Now you can compare methylation state changes in your samples without concerns over specificity, and with less time and effort than running immunoblots. Check our website regularly for new releases of Recombinant Histone H3 proteins and Histone Modification ELISAs.

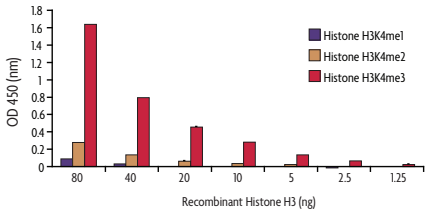


Figure 2: Histone H3 trimethyl Lys4 specificity.
1.25 to 80 ng of Recombinant Histone H3 mono-, di- and trimethyl Lys4 proteins were assayed separately using the Histone H3 trimethyl Lys4 ELISA kit. These results indicate that the Histone H3 trimethyl Lys4 detection antibody contained in Histone H3 trimethyl Lys4 ELISA kit is highly specific, with little cross-reactivity for mono- or dimethylated Lys4 on Histone H3. This means that small, specific changes in trimethyl Lys4 levels can be detected with the ELISA.

Start screening modification levels today!

Please visit us at www.activemotif.com for complete information on our Histone Modification ELISAs, modification-specific antibodies and additions to our line of Recombinant Histone H3 proteins.

Product	Format	Catalog No.
Histone H3 dimethyl Lys4 ELISA	1 x 96 rxns	53102
Histone H3 trimethyl Lys4 ELISA	1 x 96 rxns	53103

Rigorous Testing Ensures the Antibodies We Make Perform as Specified

Active Motif is committed to providing the highest quality antibodies for studying chromatin and the biology of the nucleus. We specialize in manufacturing antibodies against histones, histone modifications and chromatin proteins, many of which have been validated for use in ChIP and immunofluorescence (IF). Every antibody we make is tested rigorously to ensure you are not wasting your precious time and research dollars on antibodies that don't perform as specified. At Active Motif, we believe that you should always repeat your experiments, but not because your reagents failed.

Our Antibody Commitment

- **Quality first** – we'd rather fail our project than sacrifice quality
- **Highly characterized**– all of our antibodies are tested stringently under multiple conditions
- **Controlled process** – we manufacture and test our own antibodies
- **Consistent** – we go to great lengths to minimize lot-to-lot variability

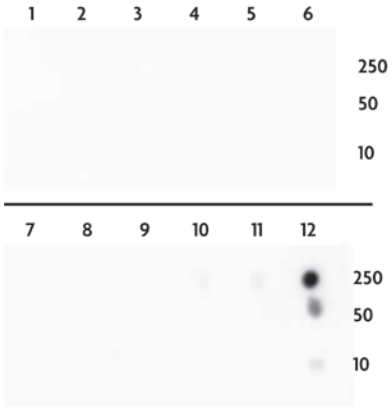


Figure 1: Dot blot analysis confirms specificity.
Ten, 50 and 250 picomole aliquots of synthetic peptides corresponding to histone H3 that is unmodified, mono-, di- or trimethylated at Lys4 (Lanes 1-4), Lys9 (Lanes 5-8) and Lys27 (Lanes 9-12) were spotted and probed with Histone H3 trimethyl Lys27 pAb (Cat. No. 39155), confirming the antibody's specificity for histone H3 trimethyl Lys27 (Lane 12). No cross-reactivity is observed for the unmodified Lys27 peptide (Lane 9) and only a hint of signal is observed at the 250 picomole spots for mono- (Lane 10) and dimethyl Lys27 (Lane 11).

Immunogen selection

Immunogens are selected to decrease the likelihood of cross-reactivity with related proteins and to maximize detection of the protein in its native context. Immunogens for modification-specific antibodies are selected to ensure that the antibody only recognizes the modified version of the protein.

Specificity screening

The first test performed on every antibody is dot blot analysis, which ensures its specificity for the desired protein or modification (Figure 1). Antibodies that do not exhibit ≥ 25-fold selectivity for the desired modification are failed.

Western blot

Western blot is carried out to verify the antibody recognizes the correct molecular weight protein and does not cross-react with other proteins. Commonly used chemical treatments that stimulate a modification (*e.g.* HDAC inhibitors and acetylation) are used to better detect the modification (Figure 2).

Technique validation

Each antibody is validated for use in techniques such as ChIP (see Page 3) and IF (Figure 3) so that you have confidence when you use them in your experiments.

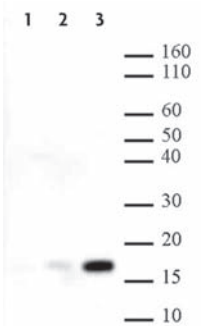


Figure 2: Western blot confirming size & modification.
Histone H2B acetyl Lys120 pAb (Cat. No. 39119) tested by Western blot. No reactivity is observed on recombinant human histone H2B (Lane 1, 200 ng protein), as it is not acetylated. Lanes 2 and 3 contain 5 µg each of HeLa cell acid extract. Cells that were treated with a histone deacetylase inhibitor (sodium butyrate) prior to extraction have an increased signal (Lane 3), as expected for an antibody to a site of acetylation.

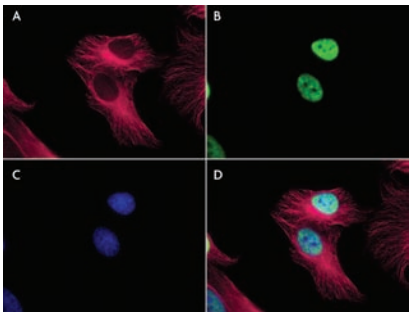


Figure 3: Immunofluorescence testing.
HeLa cells stained with alpha Tubulin mAb (Clone 5-B-1-2) in red (Cat. No. 39527, panels A and D), Histone H4 monomethyl Lys20 pAb in green (Cat. No. 39175, B and D) and DAPI in blue (C and D). Note the strong nuclear staining and absence of cytoplasmic staining in panel B, as expected of an antibody to a histone modification.

Additional specificity testing

Antibodies to histone modifications that exist in budding yeast (*S. cerevisiae*) can be additionally screened for specificity by testing strains of yeast that contain a point mutation in the modified amino acid (Figure 4).

Figure 4: Mutational analysis of yeast modifications.
Histone H4 acetyl Lys12 pAb (Cat. No. 39165) tested by Western blot using extracts from wild-type yeast or yeast containing a mutated histone H4 gene.

Lane 1: Yeast with wild-type histone H4.
Lane 2: H4 with the amino-terminal tail deleted.
Lane 3: H4 with an arginine instead of lysine 5.
Lane 4: H4 with an arginine instead of lysine 8.
Lane 5: H4 with an arginine instead of lysine 12.
Lane 6: H4 with an arginine instead of lysine 16.
Lane 7: H4 with arginines instead of lysines 5, 8 & 12.

Note the absence of reactivity in yeast extracts in which lysine 12 is either missing or mutated (Lanes 2, 5 and 7).

Why not see for yourself?

For more information, use the convenient search tools at www.activemotif.com/abs.

NEW: Assay for Nrf2 and SREBP1 Activity

TransAM™ Kits are fast and simple DNA-binding ELISAs in a convenient format with high sensitivity and specificity for over 40 targets. Now TransAM, the most published alternative to EMSA, is available for Nrf2 and SREBP1.

The TransAM method

TransAM Kits use a unique plate-based format to capture activated transcription factors, which are analyzed using an antibody specific to your isoform of interest. TransAM Kits are non-radioactive and offer up to 100-fold more sensitivity than traditional gelshift techniques, so even the smallest changes in transcription factor levels can be detected.

How it works

TransAM Kits assay for transcription factor activity by capturing the activated factor with a double-stranded oligonucleotide containing the target-specific consensus sequence that is bound to a 96 strip-well plate. When nuclear extracts from tissue or cell samples are added to the plate, the activated transcription factor binds the consensus sequence on the plate. Next, a primary antibody specific to the transcription factor is added which is subsequently detected by an HRP-conjugated secondary antibody and developed to give a colorimetric readout.

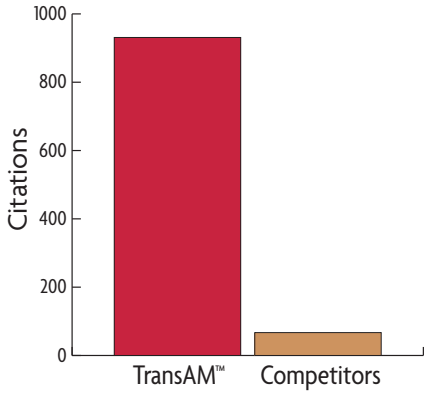


Figure 1: TransAM Kit citations.
Using HighWire Press, http://highwire.stanford.edu, a comparison was made by searching for citations of TransAM from Active Motif versus the tradenames of all competitor kits combined. TransAM is clearly the leader.

TransAM citations

TransAM is the most published ELISA assay for transcription factor activation (Figure 1). The combination of a fast, user-friendly format with high sensitivity and specificity makes it easy to see why TransAM Kits have been cited in over 900 journal articles.

TransAM Nrf2

NF-E2 related factor (Nrf2) is a critical transcription factor in oxidative stress signaling that may serve as a master regulator in cellular defense pathways, protecting a wide variety of tissues from toxic exposure. TransAM Nrf2 is a simple solution that makes studying activation of Nrf2 in human, mouse and rat samples more convenient (Figure 2).

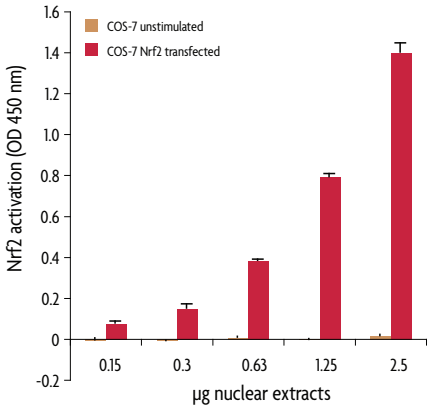


Figure 2: TransAM Nrf2 activation.
0.15 to 2.5 µg of COS-7 (Nrf2 transfected) nuclear extract (red bars) and mock-transfected COS-7 nuclear extract (copper bars) were assayed per well.

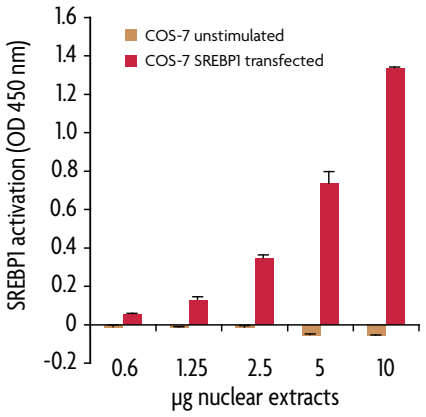


Figure 3: TransAM SREBP1 activation.
0.6 to 10 µg of COS-7 (SREBP1 transfected) nuclear extract (red bars) and mock-transfected COS-7 nuclear extracts (copper bars) were assayed per well.

TransAM SREBP1

TransAM SREBP1 (Sterol-regulatory element binding protein) detects SREBP-1a and -1c isoforms in as little as 0.6 µg nuclear extract from human, mouse and rat samples (Figure 3).

Widest selection of targets and formats

Active Motif offers the broadest selection of targets, including kits for NFκB, AP-1, STAT, PPARγ, HIF-1, NFATc1, C/EBP and many others. Please visit us at www.activemotif.com/transam for a complete product listing, and to check for the latest releases!

Product	Format	Catalog No.
TransAM™ Nrf2	1 x 96 rxns	50296
	5 x 96 rxns	50796
TransAM™ SREBP1	1 x 96 rxns	50496
	5 x 96 rxn	50996

Rapid, Sensitive Assays for HAT & HDAC Activity and Inhibitor Compounds

Active Motif's HAT & HDAC Assay Kits are easy-to-use, sensitive assays that can be used to determine the activity of histone acetyltransferases and histone deacetylases in your cell & nuclear extracts, immunoprecipitates and purified enzymes, as well as to screen the effects of potential inhibitor compounds. The HAT Assay Kit uses a fluorescent readout, while HDAC Assay Kits are available in both fluorescent and colorimetric formats.

Histone acetyltransferases (HAT) are enzymes that acetylate conserved lysine amino acids on histones. Generally, histone acetylation is associated with the activation of gene expression, as hyperacetylated chromatin is transcriptionally active. Histone deacetylases (HDAC) remove these acetyl groups from histones. Their action is opposite to that of histone acetyltransferases, as hypoacetylated chromatin is silent. Because HATs and HDACs are involved with other proteins in the regulation of gene expression, their activity is much studied, as are compounds that inhibit HAT and HDAC activity.

How does the HAT Assay Kit work?

Assaying HAT activity is easy with this 96-well plate format. Simply incubate your HAT with your choice of the provided Histone H3 or Histone H4 substrate peptides and acetyl-CoA for 10-30 minutes, then develop. The HAT Assay Kit uses a thiol-reactive fluorescent dye that reacts with the free Co-enzyme A generated by the histone acetyltransferase activity to give a fluorescent readout. This makes it easy to generate standard curves with acetyl-CoA or β-mercaptoethanol, so you can relate the fluorescence of your HAT to pmol/min/µg specific activity.

Active recombinant p300 is provided as a control for use with your samples; enough is provided so that it could also be used as a HAT to screen an entire 96-well plate of inhibitors. Anacardic acid is provided for use as a control, as it is a potent HAT inhibitor (Figure 1).

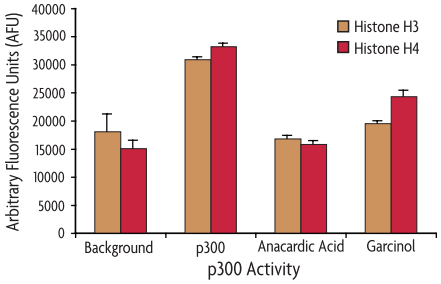


Figure 1: HAT inhibitor effects on p300 activity.
50 ng p300 were assayed per well with 50 µM acetyl-CoA and 50 µM histone H3 or H4 peptide substrates in the absence or presence of 15 µM anacardic acid or 25 µM garcinol, known HAT inhibitor compounds.

How do the HDAC Assay Kits work?

The HDAC Assay Kits utilize a peptide substrate that contains an acetylated lysine residue that can be deacetylated by Class I, II and IV HDAC enzymes. Class III HDAC enzymes, or the Sirtuins, require the addition of the NAD^+ cofactor in the assay. Once the substrate is deacetylated, the lysine reacts with the Developing Solution and releases either the chromophore or the fluorophore from the substrate to result in either a colorimetric or fluorescent product. The colorimetric product absorbs maximally at 405 nm; the fluorescent product can be read with an excitation wavelength of 360 nm and emission wavelength of 460 nm (Figure 2).

Assay for HDAC activity or inhibition

With these simple HDAC Assay Kits, you can easily assay for HDAC activity or you can screen compounds for their inhibitory effects. The provided deacetylated assay standard enables you to quantify HDAC effects with great accuracy.

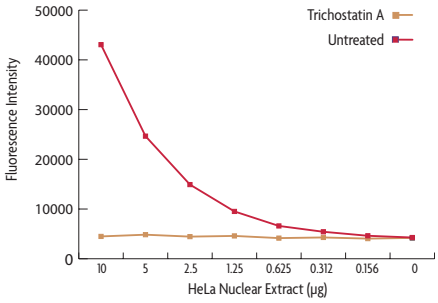


Figure 2: HDAC activity in HeLa cells.
HeLa nuclear extracts were assayed at 0 to 10 µg per well using the fluorescent version of the HDAC Assay Kit. Untreated extract results are shown with a red line, and extracts inhibited with 1 mM Trichostatin A are shown with a copper line.

Try them today!

Please give us a call or visit us at www.activemotif.com to get complete information on our HAT and HDAC Assays, as well as additional active histone acetyltransferases and related assays. Active recombinant p300 and GCN5 proteins are also available separately for use in other applications.

Product	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
Recombinant GCN5 protein, active	5 µg	31204
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

NEW: Faster Histone Purification With Convenient Spin Columns

Active Motif first brought you the ground breaking Histone Purification Kit, enabling fast and easy isolation of core histones from any cell culture or tissue sample. Now, we are expanding upon that innovative product by introducing our new spin column-based Histone Purification Mini Kit, which is even faster, more sensitive and easier to use. Purification of histone from yeast cells is also possible with this new kit. And, like the original kit, the technique ensures that post-translational modifications remain intact.

How does it work?

Our unique method utilizes a purification column with a proprietary binding matrix (Figure 1) and a series of proprietary buffers to isolate very pure fractions of histones. Using the Histone Purification Mini Kit, core histones can be purified from cultured cells, yeast cells and tissue samples as a single population containing H2A, H2B, H3 and H4 (Figure 2). The column has a high affinity for histones, so histones can be purified from as little as $8x10^5$ cells. While you can not purify the same amount of histones as with the original Histone Purification Kit, spin columns make it easier to isolate histones from multiple samples simultaneously.

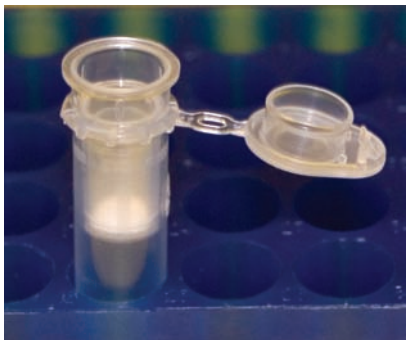


Figure 1: Histone purification in a new spin column format. Convenient spin columns enable faster purification of histones from multiple samples simultaneously.

Histone Purification Mini advantages

- Convenient spin column-based protocol
- Fast, efficient, requiring less hands on time
- Prepare histones from multiple samples simultaneously

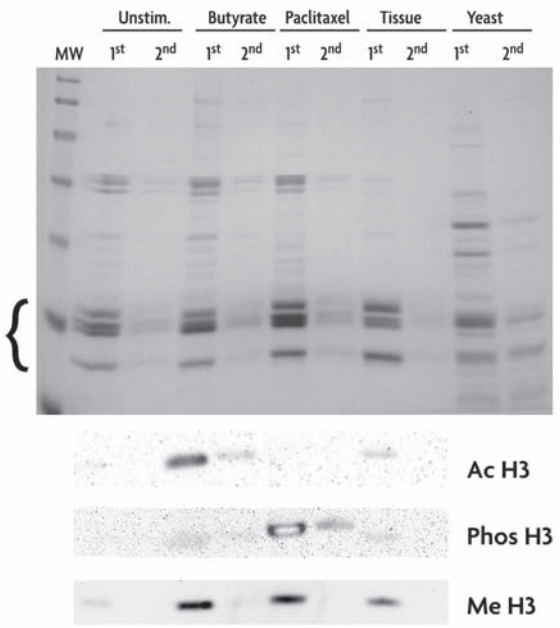


Figure 2: SDS-PAGE and Western blot analysis of histone fractions purified by the Histone Purification Mini Kit. Histones were purified from unstimulated, sodium butyrate-treated and paclitaxel-treated HeLa cells, rat brain tissue and budding yeast. The 1st and 2nd elutions from each sample were analyzed on a 16% Tris-glycine SDS-PAGE gel (top panel). The migration of the histones in the SDS gel is indicated by the bracket to the left of the gel. Westerns were then performed on the samples (bottom panel) using antibodies to acetyl-histone H3 (Ac-H3: Cat. No. 39139, 1:500 dilution), phospho-Ser28 histone H3 (Phos-H3: Cat. No. 39098, 1:1,000 dilution) and trimethyl-Lys4 histone H3 (Me-H3: Cat. No. 39159, 1:1,000 dilution).

Intact post-translational modifications

Preserving post-translational modifications like acetylation, methylation and phosphorylation is critical when investigating the role of histones in chromatin biology. Our proprietary buffer system is optimized to maintain these modifications while separating pure histone fractions (Figure 2), which can be used as substrates in many downstream assays, analyzed by Western blot or mass spectrometry, or assembled into chromatin that closely resembles native chromatin for functional assays using Active Motif's Chromatin Assembly Kit (Cat. No. 53500).

Original kit now improved

Creating the spin-column format of the new Histone Purification Mini Kit has inspired us to improve our original Histone Purification Kit, which can now be used to purify all four core histones using either the spin-column or gravity-flow format, or as separate fractions of H2A/H2B dimers and H3/H4 tetramers using the gravity-flow format.

Product	Format	Catalog No.
Histone Purification Mini Kit	20 rxns	40026
Histone Purification Kit	10 rxns	40025

Easily Compare CpG Methylation in Various DNA Samples

MethylCollector™ provides a fast, efficient protocol for isolating and comparing CpG-methylated DNA in cell or tissue samples. The kit uses a recombinant Methyl-binding protein (MBD2b) to capture DNA, rather than antibody-based immunoprecipitations, greatly improving assay sensitivity.

MethylCollector advantages

- **Rapid** – simple protocol is completed in less than 4 hours
- **Adaptable** – enables detection of samples from 5 ng to 1 µg of DNA
- **Verified** – positive control DNA and PCR primers ensure success

The MethylCollector method

In MethylCollector, His-tagged MBD2b specifically binds to CpG-methylated DNA fragments prepared by sonication or enzymatic digestion. These protein-DNA complexes are captured with nickel-coated magnetic beads and washed with a stringent high-salt buffer to remove DNA fragments with little or no methylation. Ready-to-use methylated DNA is then eluted (Figure 1). Because MethylCollector is highly efficient, you can analyze the methylation state of any specific locus on genomic DNA isolated from less than 800 cells (~5 ng DNA).

MethylCollector applications

MethylCollector enables many powerful applications, including rapid screening of the methylation status of multiple loci in tumor tissue or cells and detecting changes in DNA methylation in other situations, such as normal cellular differentiation and aging. To find out more, please visit www.activemotif.com.

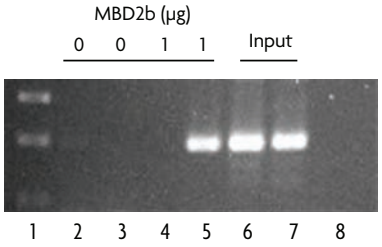


Figure 1: Isolation of Fully Methylated Jurkat DNA. MethylCollector was used to isolate methylated DNA from 100 ng genomic DNA (lanes 2 & 4) and Fully Methylated Jurkat DNA (lanes 3 & 5), with (lanes 4 & 5) or without (lanes 2 & 3) inclusion of 1 µg of the kit's His-tagged MBD2b protein. These samples and input genomic DNA (lane 6) and input Fully Methylated Jurkat DNA (lane 7) were then PCR amplified with the BRCA1 primers. A positive result is observed only with Fully Methylated Jurkat DNA captured by the kit's His-tagged MBD2b (lane 5). Lane 8 is a water-only PCR control.

Product	Format	Catalog No.
MethylCollector™	25 rxns	55002
Fully Methylated Jurkat DNA	10 µg	55003

Reproducible Bisulfite Conversion for Accurate Analysis of Methylated DNA

The MethylDetector™ Bisulfite Modification Kit makes DNA methylation analysis fast and efficient by providing optimized reagents, time-saving DNA purification columns and positive control PCR primers for assay validation.

Proven controls verify your success

DNA methylation analysis often uses bisulfite to convert unmethylated cytosines to uracils, leaving methylated cytosines unchanged. The DNA is then PCR amplified and analyzed by sequencing or restriction digest, which can be costly and time-consuming. Thus, confirming that conversion was successful before analyzing the samples is a big benefit. To that end, the MethylDetector Kit provides positive control PCR primers specific for bisulfite-converted DNA, so you can confirm the conversion worked before starting the analysis (Figure 1).

Advantages

- **Reproducible** – 99% conversion efficiency
- **Flexible** – use high G/C content sequences and uncut DNA
- **Easy** – DNA purification columns mean no more precipitations

Try MethylDetector today!

MethylDetector will speed and simplify your methylation analysis. Learn more at www.activemotif.com.

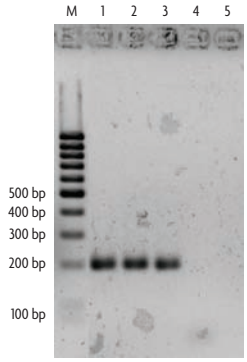


Figure 1: Reproducible conversion by MethylDetector. MethylDetector was used for bisulfite conversion of 3 different DNA samples (Lanes: 1-3) and a control with no DNA (Lane: 4). PCR was performed on these samples and an unconverted DNA control (Lane: 5) using the kit's control PCR primers. The presence of PCR product in only the converted samples demonstrates the efficiency and reproducibility of the MethylDetector Kit.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

Simple, Effective Monitoring of Phosphorylation Using In-cell Detection

Active Motif's Fast Activated Cell-based ELISA (FACE™) Kits are a simple, sensitive, cell-based method for detecting protein phosphorylation directly in the cell, without the trouble of making cell extracts or running gels.

FACE advantages

- **Cell-based** – no extraction, gels or blotting needed
- **Accurate** – fixation prevents additional protein modifications
- **Fast** – 5-hour protocol, with just 2 hours of hands-on time
- **Grow cells in 96-well plates** – save on reagents
- **Economical** – total and phospho-specific antibodies provided for 2 x 96 wells
- **Semi-quantitative results** – normalize to total protein and cell number

The "in-cell" method

Fast Activated Cell-based ELISAs (FACE™) are easy to use and require just 2 hours of hands-on time. Cells are grown in 96-well cell culture plates and treated to induce phosphorylation of the protein of interest. The cells are rapidly fixed with formaldehyde to preserve the phosphorylation state, and then each well is incubated with a primary antibody specific for either the total protein or its phosphorylated form. Subsequent incubation with a labeled secondary provides a colorimetric or chemiluminescent readout that is quantitative and reproducible (Figures 1 & 2). Data is easily normalized to cell number using the provided Crystal Violet Dye.

A variety of kits to choose from

FACE Kits are available for over 20 different targets (Table 1). The Suspension Cell FACE module was designed to work with all FACE Kits; it improves results when working with suspension cells by providing 96-well filter plates that make it easier to perform washing & liquid handling steps. And, with FACE Maker Kits, you can use your own primary and secondary antibodies to detect any target or modification state of interest.

Simplify your phospho-assays today!

For complete information on the FACE product line, please give us a call or visit us at www.activemotif.com/face.

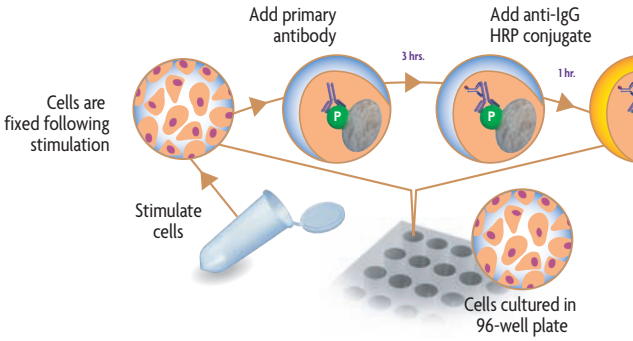


Figure 1: Flow chart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects total protein as well as the phosphorylated form of the protein.

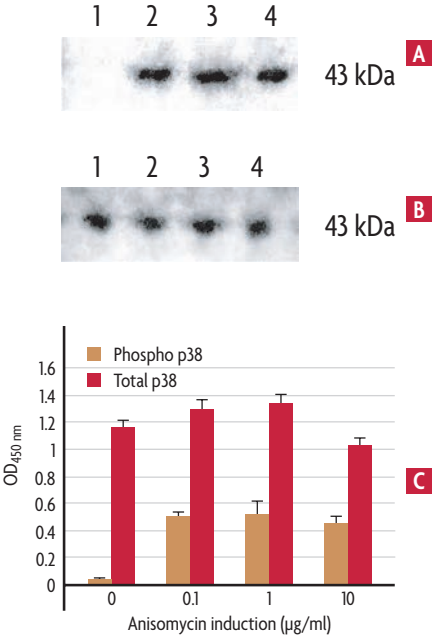


Figure 2: Phospho and total p38 MAPK assays.
Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 antibodies (B). For FACE, 4/4 cells were grown in 96-well plates, stimulated as above, fixed and then assayed in triplicate using the FACE p38 Kit (C). Data were corrected for cell number through use of the kit's Crystal Violet Dye. Western blot data provided courtesy of Dr. Henri H. Versteeg and Dr. Maikel P. Peppelenbosch.

Table 1: The FACE™ Product Line

FACE™ AKT (S473)	FACE™ ATF-2 (T71)	FACE™ Bad (S112)	FACE™ c-Jun (S63)	FACE™ c-Jun (S73)	FACE™ c-Src (Y418)
FACE™ EGFR (Y845)	FACE™ EGFR (Y992)	FACE™ EGFR (Y1173)	FACE™ ErbB-2 (Y877)	FACE™ ErbB-2 (Y1248)	FACE™ ERK1/2 (T202/Y204 & T185/Y187)
FACE™ FAK (Y397)	FACE™ FKHR (FOXO1) (T24)	FACE™ GSK3β (S9)	FACE™ HSP27 (S82)	FACE™ JAK1 (Y1022/Y1023)	FACE™ JNK (T183/Y185)
FACE™ MEK1/2 (S217/S221)	FACE™ NFκB Profiler (S468 & S536)	FACE™ p38 (T180/Y182)	FACE™ PI3 Kinase p85	FACE™ STAT2 (Y869)	FACE™ STAT4 (Y693)
FACE™ STAT6 (Y641)	FACE™ Maker	Suspension Cell FACE™			

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- Rapid, Sensitive Assays for HAT & HDAC Activity and Inhibitor Compounds
- Faster Histone Purification with Convenient Spin Columns
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- Reproducible Bisulfite Conversion for Analysis of Methylated DNA
- Simple, Effective Monitoring of Phosphorylation Using In-cell Detection

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