Histone H3 dimethyl Lys9 ELISA

Catalog No. 53108

Histone H3 trimethyl Lys9 ELISA

Catalog No. 53109

(version C1)

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Overview

The addition or removal of modifications such as phospho-, methyl- and acetyl- functional groups to histones can have a profound effect on nuclear signaling as these dynamic modifications are critical in the regulation of transcription, chromosome packaging, DNA damage repair and functional genomics. Screening extracts for specific histone modifications is a simple way to assess cell health and the effect of treatment compounds on cell division.

The Histone H3 methylated Lys9 ELISAs are a simple solution for screening levels of di- or trimethylated lysine 9 on histone H3 in human, mouse and rat systems. These kits are sandwich ELISAs that utilize a Histone H3 monoclonal antibody to capture histone H3 from your samples and a rabbit polyclonal Histone H3 di- or trimethyl Lys9 antibody for specific detection. A secondary antibody conjugated to horseradish peroxidase (HRP) and developing solutions provide a sensitive colorimetric readout that is easily quantified by spectrophotometry. The assay is performed in a convenient 96-stripwell plate, enabling you to simultaneously screen from 1 to 96 samples in a single experiment. It works with acid extracts from tissue or cell samples and is able to detect methylated histone H3 on lysine 9 in as little as 15 nanograms of core histone preparations and 5 micrograms of acid extract.

For added convenience and a more quantitative interpretation of results, the histone methylation ELISA kits all include Active Motif's recombinant methylated histone technology. Each methylated histone ELISA kit is supplied with the a 99% pure Histone H3 recombinant protein that has been specifically methylated at the desired lysine site. The included Recombinant Histone H3 di- or trimethyl Lys9 protein enables you to build a reference standard curve to quantitate the amount of specifically methylated H3 Lys9 in your samples.

product	format	catalog no.
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109

Introduction

Histone H3 methylated Lys9

The basic structural unit of chromatin is the nucleosome, which consists of 146 base pairs (bp) of DNA wrapped around a histone octamer. The histone octamer consists of two copies each of the core histone H2A-H2B dimers and a tetramer of H3-H4. A linker histone, histone H1, binds chromatin outside the nucleosome unit to regulate chromatin structure.

Histone modifications such as phosphorylation, acetylation and methylation at specific amino acid residues on the histone tails that extend beyond the core nucleosome have been found to influence and regulate transcription, chromosome packaging and DNA damage repair. Many of these specific histone modifications are conserved throughout eukaryotes. While the biological significance of some histone modifications remains to be understood, some have been demonstrated to correlate very closely with specific cellular states like transcriptional activity[1,2].

The methylation of lysine 9 (K9) on histone H3 has been correlated with the regulation of gene transcription[1,2,3], making methylated lysine 9 on histone H3 a significant marker in studying the state of transcription activity. Methylation of histone H3 at lysine 9 is linked to gene silencing and transcriptional repression. Methyltransferases G9a, G9a-related protein (GLP) and SUV39H1 are believed to have specificity for methylation of lysine 9 on histone H3[4].

Active Motif's Histone Modification ELISA Kits make it is easy to screen for changes in methylation levels. Histone H3 di- or trimethyl Lys9 ELISA works with acid extracts from tissue or cell samples as well as purified core histones, such as those isolated using Active Motif's Histone Purification Kits (Catalog Nos. 40025 & 40026). The sensitive, specific assays are able to detect methylated lysine 9 on histone H3 in less than 3.5 hours. As this assay is performed in a 96-stripwell plate, a large number of samples can be handled simultaneously, allowing for high-throughput automation.

Histone Modification ELISAs have many applications including screening the effects of compounds on the methylation levels of histone H3 lysine residues.

Kit Performance and Benefits

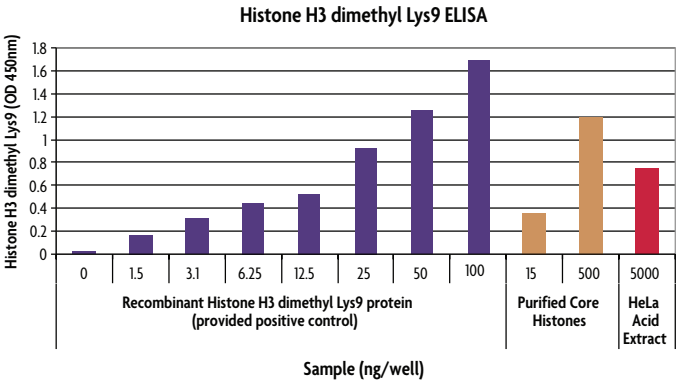
Detection limit: > 15 ng/well of purified core histones. For acid extracts, > 5 µg/well is recommended.

Range of detection: This ELISA provides quantitative results from 15 ng to 1 µg of purified core histones or from 5 µg to 20 µg of histones isolated by acid extraction. The linear range of the provided Recombinant Histone H3 dimethyl Lys9 protein is approximately from 1.5 to 50 ng/well. The linear range of the provided Recombinant Histone H3 trimethyl Lys9 protein is approximately from 15 to 250 ng/well.

Cross-reactivity: Human, mouse, rat, yeast, and a wider range of species reactivity is predicted due to the high degree of sequence homology of histone H3.

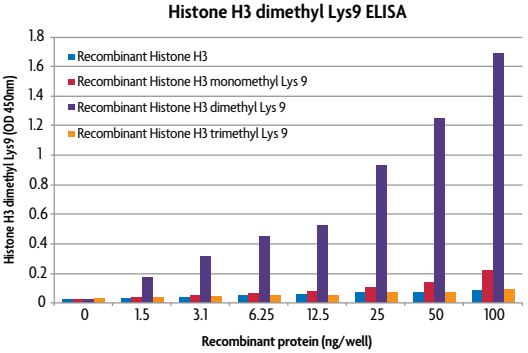
Assay time: 3.5 hours.

Histone H3 dimethyl Lys9 ELISA Kit



Histone H3 dimethyl Lys9 detection.

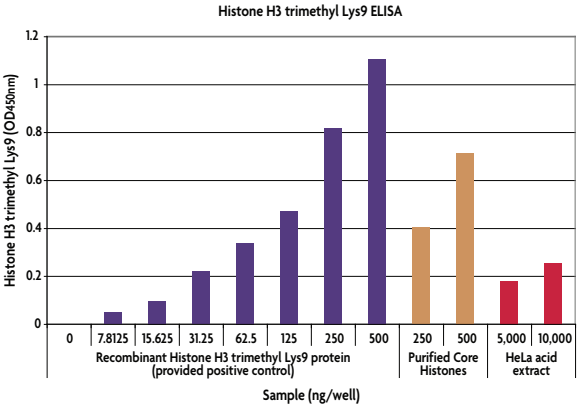
The Histone H3 dimethyl Lys9 ELISA was used to assay purified HeLa core histones (15 & 500 ng) made using Active Motif's Histone Purification Mini Kit (Catalog No. 40026) and HeLa acid extracts (5 µg) prepared as stated in Appendix Section A. The provided Recombinant Histone H3 dimethyl Lys9 protein was assayed from 1.5 - 100 ng/well as a reference standard curve. Data shown are the results from wells assayed in duplicate. These results are provided for demonstration only.



Histone H3 dimethyl Lys9 specificity.

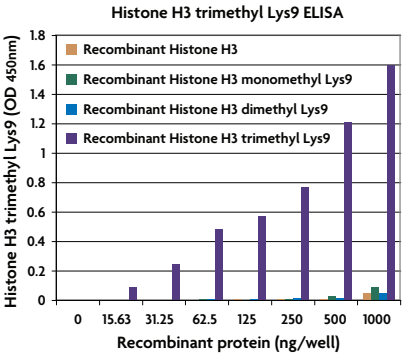
Recombinant Histone H3, mono-, di- and trimethyl Lys9 proteins were assayed from 1.5 ng - 100 ng per well using the Histone H3 dimethyl Lys9 ELISA. These results indicate the specificity of the assay. There is extremely low background from histone H3 and little cross-reactivity for mono- or trimethyl Lys9. This means that small, specific changes in dimethyl Lys9 levels can easily be detected with this kit.

Histone H3 trimethyl Lys9 ELISA Kit



Histone H3 trimethyl Lys9 detection.

The Histone H3 trimethyl Lys9 ELISA was used to assay purified HeLa core histones (250 - 500 ng) made using Active Motif's Histone Purification Mini Kit (Catalog No. 40026) and HeLa acid extracts (5 -10 µg) prepared as stated in Appendix Section A. The provided Recombinant Histone H3 trimethyl Lys9 protein was assayed from 7.8125 - 500 ng/well as a reference standard curve. Data shown are the results from wells assayed in duplicate. These results are provided for demonstration only.



Histone H3 trimethyl Lys9 specificity.

Recombinant Histone H3, mono-, di- and trimethyl Lys9 proteins were assayed from 15 ng - 1 µg per well using the Histone H3 trimethyl Lys9 ELISA. These results indicate the specificity of the assay. There is extremely low background from histone H3 and little cross-reactivity for mono- or dimethyl Lys9. This means that small, specific changes in trimethyl Lys9 levels can easily be detected with this kit.

Kit Components and Storage

Histone H3 methylated Lys9 ELISA Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
Histone H3 di- or trimethyl Lys9 antibody	6 µl	4°C
HRP-conjugated anti-rabbit IgG	6 µl	4°C
Assay Dilution Buffer	15 ml	4°C
20X Wash Buffer	25 ml	4°C
Developing Solution	11 ml	4°C
Stop Solution	11 ml	4°C
Recombinant Histone H3 di- or trimethyl Lys9	10 µg (1 µg/µl)	-80°C
Histone H3 Capture Plate	1	4°C
Plate sealer	1	RT

Additional materials required

- Histone samples (recombinant, purified or acid extracted)
- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Rocking platform/orbital shaker
- Microplate spectrophotometer capable of reading at 450 nm (655 nm as optional reference wavelength)

Buffer Preparation and Recommendations

Assay Dilution Buffer

Assay Dilution Buffer is provided as a 1X solution and is ready for use once thawed.

20X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay as follows: For every 100 ml of 1X Wash Buffer required, dilute 5 ml 20X Wash Buffer with 95 ml sterile water (see the Quick Chart for Preparing Buffers in this section). Mix gently to avoid foaming. The 1X Wash Buffer may be stored at 4°C for one week. The Tween 20 contained in the 20X Wash Buffer may form clumps, therefore it is necessary to completely resuspend any precipitates by incubating at 50°C for 2 minutes and mixing prior to use.

Preparation of antibodies (See the Quick Chart for Preparing Buffers in this Section.)

Dilute the Histone H3 dimethyl antibody 1:2000 with Assay Dilution Buffer. Use 50 µl per well.
Dilute the Histone H3 trimethyl antibody 1:1000 with Assay Dilution Buffer. Use 50 µl per well.

Dilute the HRP-conjugated anti-rabbit IgG antibody 1:2000 with Assay Dilution Buffer. Use 50 µl per well.

Developing Solution

The Developing Solution should be warmed to room temperature before use. The Developing Solution is light sensitive, therefore, we recommend avoiding direct exposure to intense light during storage. The Developing Solution may develop a yellow hue over time. This does not affect product performance. However, a blue color present in the Developing Solution indicates that it has been contaminated and must be discarded. Prior to use, place the Developing Solution at room temperature for at least 1 hour. Transfer the amount of Developing Solution required for the assay into a secondary container before aliquoting into the wells (see the Quick Chart for Preparing Buffers in this section). After use, discard remaining Developing Solution.

Stop Solution

Prior to use, transfer the amount of Stop Solution required for the assay into a secondary container (see the Quick Chart for Preparing Buffers in this section). After use, discard remaining Stop Solution.

WARNING: The Stop Solution is corrosive. Wear personal protective equipment when handling, *i.e.* safety glasses, gloves and labcoat.

Diluting Recombinant Histone H3 methylated Lys9 proteins

The Recombinant Histone H3 di- or trimethyl Lys9 protein is provided as a control for quantitating the amount of methylated histone H3. There is enough recombinant protein for at least 2 standard curves. During the first use, we recommend making either 3 µl or 6 µl aliquots of the stock protein and storing at -80°C to avoid multiple freeze/thaw cycles.

Preparing histone samples

Histone samples can be prepared using several techniques:

1. A simple acid extraction (see Appendix Section A) is recommended instead of a nuclear extraction as histones are soluble in acidic solutions and many nuclear extraction procedures often exclude histones from the final sample. The acid extraction will provide crude histones.
2. Purified core histones, such as those obtained from Active Motif's Histone Purification Kits (Catalog Nos. 40025 & 40026) produce distinct, clean core histone samples as determined by gel electrophoresis.
3. More stringent purification techniques use a hydroxyapatite column to provide highly pure, core histone samples, such as Active Motif's HeLa core Histones (Catalog No. 53501).

Regardless of the histone preparation technique, it is recommended initially to use a range of sample concentrations (*e.g.* 5 ng, 50 ng, 500 ng, 5 µg) in order to determine the amount of sample necessary to fall within the linear area of the reference curve. Once the protein concentration for the linear area of the reference curve has been determined, perform the rest of the assays within the linear range.

Quick Chart for Preparing Buffers

Reagents to prepare	Components	For 1 well	For 1 strip (8 wells)	For 6 strips (48 wells)	For 12 strips (96 wells)
Primary Antibody	Histone H3 dimethyl Lys9 Ab	0.03 µl	0.23 µl	1.4 µl	2.7 µl
	Assay Dilution Buffer	51.97 µl	450 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
	Histone H3 trimethyl Lys9 Ab	0.05 µl	0.45 µl	2.7 µl	5.4 µl
	Assay Dilution Buffer	51.95 µl	450 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
Secondary Antibody	HRP-conjugated anti-rabbit IgG	0.026 µl	0.23 µl	1.35 µl	2.7 µl
	Assay Dilution Buffer	52 µl	450 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
1X Wash Buffer	Distilled water	1.9 ml	0.9 ml	95 ml	190 ml
	20X Wash Buffer	100 µl	17.1 ml	5 ml	10 ml
	TOTAL REQUIRED	**2 ml**	**18 ml**	**100 ml**	**200 ml**
Developing Solution	**TOTAL REQUIRED**	**112.5 µl**	**900 µl**	**5.4 ml**	**10.8 ml**
Stop Solution	**TOTAL REQUIRED**	**112.5 µl**	**900 µl**	**5.4 ml**	**10.8 ml**

Read the entire protocol before use.

Determine the appropriate number of microwell strips required for testing samples, controls and blanks in duplicate. Store the unused strips in the aluminum pouch at 4°C. If less than 8 wells in a strip need to be used, cover the unused wells with a portion of the plate sealer while you perform the assay. The unused wells are stable at room temperature for the duration of the assay if kept dry. Once the assay is finished, unused strips should be returned to the aluminum pouch and stored at 4°C for a separate assay. Use the strip holder while performing the assay.

Prepare the 1X Wash Buffer as described above in the section Buffer Preparation and Recommendations. Multi-channel pipettor reservoirs may be used for dispensing the Wash Buffer, Assay Dilution Buffer, Developing Solution and Stop Solution into the wells being used.

Standard Curve Preparation for H3 dimethyl Lys9

Use this plate set-up example to prepare a standard curve for the Histone H3 dimethyl Lys9 (H3 K9me2) Kit in duplicate.

	H3 K9me2											
	1	2	3	4	5	6	7	8	9	10	11	12
A	100 ng	100 ng	–	–	–	–	–	–	–	–	–	–
B	50 ng	50 ng	–	–	–	–	–	–	–	–	–	–
C	25 ng	25 ng	–	–	–	–	–	–	–	–	–	–
D	12.5 ng	12.5 ng	–	–	–	–	–	–	–	–	–	–
E	6.25 ng	6.25 ng	–	–	–	–	–	–	–	–	–	–
F	3.125 ng	3.125 ng	–	–	–	–	–	–	–	–	–	–
G	1.56 ng	1.56 ng	–	–	–	–	–	–	–	–	–	–
H	0 ng	0 ng	–	–	–	–	–	–	–	–	–	–

1. Recombinant Histones are provided at a 1 µg/µl concentration. Thaw the protein on ice. Before using, vortex to the tube for 10 seconds and quick spin the contents to the bottom of the tube. During the first use we recommend making 3 µl aliquots of the stock protein for future standard curves and storing at -80°C to avoid multiple freeze/thaw cycles.

   Dilute the Recombinant Histone H3 dimethyl Lys9 to a starting concentration of 2 ng/µl by adding 2 µl of Recombinant Histone H3 dimethyl Lys9 to 998 µl of Assay Dilution Buffer. The result will be 2 µg/1000 µl = 2 ng/µl. Mix well by vortexing.
2. Add 100 µl of the diluted Recombinant Histone to wells A1 and A2. Discard any unused diluted Recombinant Histone.
3. Add 50 µl of Assay Dilution Buffer to wells B1 through H2.

4. Perform a serial two-fold dilution of the extracts by transferring 50 µl of the extracts in row A to the wells in row B.
5. Mix the contents of row B by pipetting up and down 3-5 times. Do not change pipette tips between well transfers.
6. Transfer 50 µl of the contents of row B to row C and mix, as previously described.
7. Continue this process until row G is reached.
8. When row G is reached, discard 50 µl of the well contents so that the final volume is 50 µl.
9. Row H will serve as the blank wells.

Standard Curve Preparation for H3 trimethyl Lys9

Use this plate set-up example to prepare a standard curve for the Histone H3 trimethyl Lys9 (H3 K9me3) Kit in duplicate.

	H3 K9me3											
	1	2	3	4	5	6	7	8	9	10	11	12
A	500 ng	500 ng	–	–	–	–	–	–	–	–	–	–
B	250 ng	250 ng	–	–	–	–	–	–	–	–	–	–
C	125 ng	125 ng	–	–	–	–	–	–	–	–	–	–
D	62.5 ng	62.5 ng	–	–	–	–	–	–	–	–	–	–
E	31.25 ng	31.25 ng	–	–	–	–	–	–	–	–	–	–
F	15.6 ng	15.6 ng	–	–	–	–	–	–	–	–	–	–
G	7.8 ng	7.8 ng	–	–	–	–	–	–	–	–	–	–
H	0 ng	0 ng	–	–	–	–	–	–	–	–	–	–

1. Recombinant Histones are provided at a 1 µg/µl concentration. Thaw the protein on ice. Before using, vortex to the tube for 10 seconds and quick spin the contents to the bottom of the tube. During the first use we recommend making 3 µl aliquots of the stock protein for future standard curves and storing at -80°C to avoid multiple freeze/thaw cycles.

   Dilute the Recombinant Histone H3 trimethyl Lys9 to a starting concentration of 10 ng/µl by adding 2 µl of Recombinant Histone H3 trimethyl Lys9 to 198 µl of Assay Dilution Buffer. The result will be 2 µg/200 µl = 10 ng/µl. Mix well by vortexing.
2. Add 100 µl of the diluted Recombinant Histone to wells A1 and A2. Discard any unused diluted Recombinant Histone.
3. Add 50 µl of Assay Dilution Buffer to wells B1 through H2.
4. Perform a serial two-fold dilution of the extracts by transferring 50 µl of the extracts in row A to the wells in row B.

5. Mix the contents of row B by pipetting up and down 3-5 times. Do not change pipette tips between well transfers.
6. Transfer 50 µl of the contents of row B to row C and mix, as previously described.
7. Continue this process until row G is reached.
8. When row G is reached, discard 50 µl of the well contents so that the final volume is 50 µl.
9. Row H will serve as the blank wells.

Step 1: Binding of H3 to the Capture Plate

1. In duplicate, prepare the amount of desired sample. It is recommended to try a range of concentrations in order to determine the amount of sample necessary to fall within the linear range of the reference curve. Add desired amount of sample in 50 µl volume to plate.

   Purified core histones: Recommended range of 15 ng - 1 µg

   Acid extracts: Recommended range of 5 - 20 µg
2. Incubate plate containing the protein standard curve and samples for 1 hour at room temperature with agitation on orbital shaker or rocking platform.
3. After the incubation, wash the wells 3 times with 200 µl of Wash Buffer.

Step 2: Binding of Primary Antibody

4. Dilute the Histone H3 dimethyl Lys9 antibody 1:2000 in Assay Dilution Buffer and mix thoroughly. Dilute the Histone H3 trimethyl Lys9 antibody 1:1000 in Assay Dilution Buffer and mix
   thoroughly.
5. Add 50 µl of diluted primary antibody to each well.
6. Incubate at room temperature for 1 hour with agitation.
7. After the incubation, wash the wells 3 times with 200 µl of Wash Buffer.

Step 3: Binding of Secondary Antibody

8. Dilute the HRP-conjugated anti-rabbit IgG antibody 1:2000 in Assay Dilution Buffer and mix thoroughly.
9. Add 50 µl of the diluted secondary antibody solution to each well.
10. Incubate at room temperature for 1 hour without agitation.
11. During this incubation, place the Developing Solution at room temperature.
12. After the incubation, wash the wells 3 times with 200 µl of wash buffer.

Step 4: Colorimetric Reaction

13. Remove as much of the final wash as possible by blotting the plate on paper towels.
14. Add 100 µl of room temperature Developing Solution to all wells being used.
15. Incubate under low light conditions from 30 seconds to 5 minutes at room temperature protected from direct light. Please read the Certificate of Analysis supplied with this kit for optimal development time associated with this lot number. Monitor the blue color development in the protein standard curve wells containing the higher concentrations of Recombinant Histone H3 di- or trimethyl Lys9 protein until they turn medium to dark blue. Do not overdevelop.
16. Add 100 µl of Stop Solution to all the wells. In presence of the acid, the blue color turns yellow.
17. Read absorbance on a spectrophotometer within 5 minutes at 450nm with an optional reference wavelength of 655 nm. Blank the plate reader according to the manufacturer's instructions using the blank wells.

    Reading the reference wavelength is optional. Most microtiter plate readers are equipped to perform dual wavelength analysis and with the appropriate software, will automatically subtract the reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, you may read the plate twice, once at 450 nm and once at 655 nm then manually subtract the 655 nm OD from the 450 nm OD values.

Calculation of results using the standard curve

To generate a standard curve using the included Recombinant Histone H3 di- or trimethyl Lys9 protein, average the duplicate readings for each standard, control, and sample and subtract the optical density (OD) obtained from the zero standard (Row H blank wells).

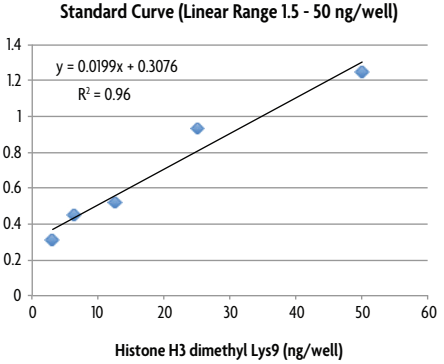
Plot the OD for the standards against the quantity (ng/well) of the standards and draw the best fit curve. The best curve fit may vary depending on the developing times used and should be calculated each time a standard curve is run. Recombinant Histone H3 dimethyl Lys9 protein has an approximate linear range from 1.5 to 50 ng. Recombinant Histone H3 trimethyl Lys9 protein has an approximate linear range from 15 to 250 ng. The data can be linearized using log/log paper and regression analysis may also be applied.

To quantify the amount of di- or trimethyl Lys9 from histone H3 in the samples, find the absorbance value for the samples on the y-axis and extend a horizontal line to the standard curve. At the intersection point extend a vertical line to the x-axis and read the corresponding standard value. Note: If the samples have been diluted, the value read from the standard curve must be multiplied by the dilution factor.

See the example standard curve on the next page.

Example curve:

The following standard curve is provided for demonstration only. A standard curve should be made every time an experiment is performed.



References

1. Kirmizis, A., *et al.* (2004) *Genes & Dev.* 18: 1592-1605.
2. Squazzo, S., *et al.* (2006) *Genome Res.* 16: 890-900.
3. Rinn, J., *et al.* (2007) *Cell* 129(7):1311-1323.
4. Zhang, Y. and Martin, C. (2005) *Nature* 6: 838-849.

Appendix

Section A. Preparation of Acid Extract/Crude histone proteins

This procedure can be used for a confluent cell layer of 150 mm plate. The yield is approximately 0.15 mg of nuclear proteins from 9 x 10^6 cells.

1. Grow HeLa cells to 70% confluency in DMEM with 10% FBS.
2. Treat cells as desired.
3. Wash cells with 1X PBS and aspirate.
4. Add 3-5 ml of PBS per 150 mm plate.
5. Scrape cells from the plate and transfer to a 50 ml conical tube.
6. Pellet the cells by centrifugation in a pre-cooled 4°C rotor at 200 x g for 5-10 minutes.
7. Aspirate as much of the PBS as possible without disturbing the cell pellet.
8. Resuspend the cell pellet in 5 volumes of Lysis Buffer (see below).
9. Incubate on ice for 30 minutes and occasionally invert the tube to mix.
10. Centrifuge the lysate at 11,000 x *g* for 10 minutes at 4°C.
11. Collect the supernatant fraction containing acid soluble proteins, and discard the acid-insoluble pellet.
12. Immediately neutralize the acid extracted proteins by adding 2/5 the total volume of Neutralization Buffer (see below).
13. Quantify the protein concentration of your acid extraction using either gel electrophoresis or a Bradford Assay.

    Gel electrophoresis is a more sensitive technique to determine histone concentration as histones are most effectively stained by Coomassie dye in a gel matrix. To determine the protein concentration run a BSA or histone standard curve on the gel.

    A Bradford Assay can be used to determine total protein concentration, not just the concentration of crude histone proteins. A total protein determination, however, is sufficient for use in the Histone Modification ELISA Kits. The quantity of acid extract tested in the Histone Modification ELISA Kits are based on total protein determination values.
14. Immediately aliquot the extract in small volumes to avoid multiple freeze/thaws.
15. Store the protein at -80°C for long-term stability.

Lysis Buffer:

0.4 M HCl

Neutralization Buffer:

1 M Sodium phosphate, dibasic, pH 12.5. Use 5 M NaOH to adjust the pH.
2.5 mM DTT
10 mM PMSF

Note: DTT and PMSF must be added immediately prior to use.

Section B: Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in all wells in the correct order
	Substrate or conjugate is no longer active	Test conjugate and substrate for activity by mixing a small aliquot of HRP and Developing Solution together
	Enzyme inhibitor present	Sodium azide will inhibit the peroxidase reaction. Follow our recommendations to prepare buffers
	Plate reader settings not optimal	Verify the wavelength and filter settings in the plate reader
	Incorrect assay temperature	Bring Developing Solution and Stop Solution to room temperature before using
	Inadequate volume of Developing Solution	Check to make sure that correct volume is delivered by pipette
High background in all wells	Developing time too long	Stop enzymatic reaction as soon as the positive wells turn medium-dark blue
	Concentration of antibodies is too high	Increase antibody dilutions
	Inadequate washing	Ensure all wells are filled with Wash Buffer and follow washing recommendations
Uneven color development	Incomplete washing of wells	Ensure all wells are filled with Wash Buffer and follow washing recommendations
	Well cross-contamination	Follow washing recommendations
High background in sample wells	Too much sample per well	Decrease amount of sample per well. For acid extracts, dilute down to 5-10 µg/well and for purified core histone, dilute down to 100 ng/well
	Concentration of antibodies is too high	Perform antibody titration to determine optimal working concentration. Start using 1:2000 for primary antibody and 1:5000 for the secondary antibody. The sensitivity of the assay will be decreased
No signal or weak signal in sample wells	Not enough sample per well	For purified core histones, increase to 500 ng per well. For extracts, make sure you are using an acid extract by following the protocol recommended in Appendix Section A. Increase amount of acid extract to 10 µg/well
No signal or weak signal in standard curve wells	Too many freeze/thaw cycles of protein	During the kit's first use, aliquot the stock recombinant protein control into 3 - 6 µl aliquots and store at -80°C to avoid multiple freeze/thaws

Section C. Related Products

Histone ELISAs	Format	Catalog No.
Histone H3 monomethyl Lys4 ELISA	1 x 96 rxns	53101
Histone H3 dimethyl Lys4 ELISA	1 x 96 rxns	53112
Histone H3 trimethyl Lys4 ELISA	1 x 96 rxns	53113
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 monomethyl Lys27 ELISA	1 x 96 rxns	53104
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100
Total Histone H3 ELISA	1 x 96 rxns	53110

Recombinant Methylated Histones	Format	Catalog No.
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H2B	50 µg	31252
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
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	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Control Acid Extracts	Format	Catalog No.
HeLa acid extract	100 µg	36200
HeLa acid extract (Paclitaxel treated)	100 µg	36201
HeLa acid extract (Sodium Butyrate treated)	100 µg	36202
HeLa acid extract (Etoposide treated)	100 µg	36203
HeLa acid extract (Anacardic acid treated)	100 µg	36204

DNA Methylation	Format	Catalog No.
MethylDetector™	50 rxns	55001
MethylCollector™	25 rxns	55002
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
Fully Methylated Jurkat DNA	10 µg	55003

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

Chromatin Assembly	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

Histone Acetyltransferase and Deacetylase Activity	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Co-Immunoprecipitation	Format	Catalog No.
Nuclear Complex Co-IP Kit	50 rxns	54001
Universal Magnetic Co-IP Kit	25 rxns	54002

SUMOylation	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express HT	96 rxns	53018
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™	25 rxns	53001
ChIP-IT™ w/o controls	25 rxns	53004
ChIP-IT™ Shearing Kit	10 rxns	53002
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Enzymatic w/o controls	25 rxns	53007
Enzymatic Shearing Kit	10 rxns	53005
Salmon Sperm DNA/Protein G agarose	25 rxns	53003
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021

Active Motif also offers a growing list of application validated antibodies, including antibodies for histones and histone modifications, transcription factor antibodies, DNA methylation-related antibodies and ChIP validated antibodies. For a complete list go to **www.activemotif.com/abs**

Transcription Factor ELISAs	Format	Catalog No.
TransAM™ AML-1/Runx1	1 x 96-well plate	47396
TransAM™ AML-3/Runx2	1 x 96-well plate	44496
TransAM™ AP-1 Family	2 x 96-well plates	44296
TransAM™ AP-1 c-Fos	1 x 96-well plate	44096
TransAM™ AP-1 c-Jun	1 x 96-well plate	46096
TransAM™ AP-1 FosB	1 x 96-well plate	45096
TransAM™ AP-1 JunD	1 x 96-well plate	43496
TransAM™ ATF-2	1 x 96-well plate	42396
TransAM™ c-Myc	1 x 96-well plate	43396
TransAM™ C/EBP α/β	1 x 96-well plate	44196
TransAM™ CREB	1 x 96-well plate	42096
TransAM™ pCREB	1 x 96-well plate	43096
TransAM™ Elk-1	1 x 96-well plate	44396
TransAM™ ER	1 x 96-well plate	41396
TransAM™ FKHR (FOXO1/4)	1 x 96-well plate	46396
TransAM™ GATA Family	2 x 96-well plates	48296
TransAM™ GATA-4	1 x 96-well plate	46496
TransAM™ GR	1 x 96-well plate	45496
TransAM™ HIF-1	1 x 96-well plate	47096
TransAM™ HNF Family	2 x 96-well plates	46296
TransAM™ HNF-1	1 x 96-well plate	46196
TransAM™ IRF-3 (Human)	1 x 96-well plate	48396
TransAM™ IRF-3 (Mouse)	1 x 96-well plate	48496
TransAM™ IRF-7	1 x 96-well plate	50196
TransAM™ MAPK Family	2 x 96-well plates	47296
TransAM™ MEF2	1 x 96-well plate	43196
TransAM™ MyoD	1 x 96-well plate	47196
TransAM™ NF-YA	1 x 96-well plate	40396
TransAM™ NFATc1	1 x 96-well plate	40296
TransAM™ NFκB Family	2 x 96-well plates	43296
TransAM™ Flexi NFκB Family	2 x 96-well plates	43298
TransAM™ NFκB p50	1 x 96-well plate	41096
TransAM™ NFκB p50 Chemi	1 x 96-well plate	41097
TransAM™ Flexi NFκB p50	1 x 96-well plate	41098
TransAM™ NFκB p52	1 x 96-well plate	48196
TransAM™ NFκB p52 Chemi	1 x 96-well plate	48197
TransAM™ NFκB p65	1 x 96-well plate	40096
TransAM™ NFκB p65 Chemi	1 x 96-well plate	40097
TransAM™ Flexi NFκB p65	1 x 96-well plate	40098
TransAM™ Nrf2	1 x 96-well plate	50296
TransAM™ Oct-4	1 x 96-well plate	42496
TransAM™ p53	1 x 96-well plate	41196
TransAM™ PPARγ	1 x 96-well plate	40196
TransAM™ Sp1	1 x 96-well plate	41296
TransAM™ Sp1/Sp3	1 x 96-well plate	40496
TransAM™ STAT Family	2 x 96-well plates	42296
TransAM™ STAT3	1 x 96-well plate	45196
TransAM™ T-bet	1 x 96-well plate	51396

For a complete, up-to-date list of available TransAM™ Kits, please visit **www.activemotif.com/transam**

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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