Histone H3 acetyl Lys27 ELISA

(version A2)

Catalog No. 53116

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Overview

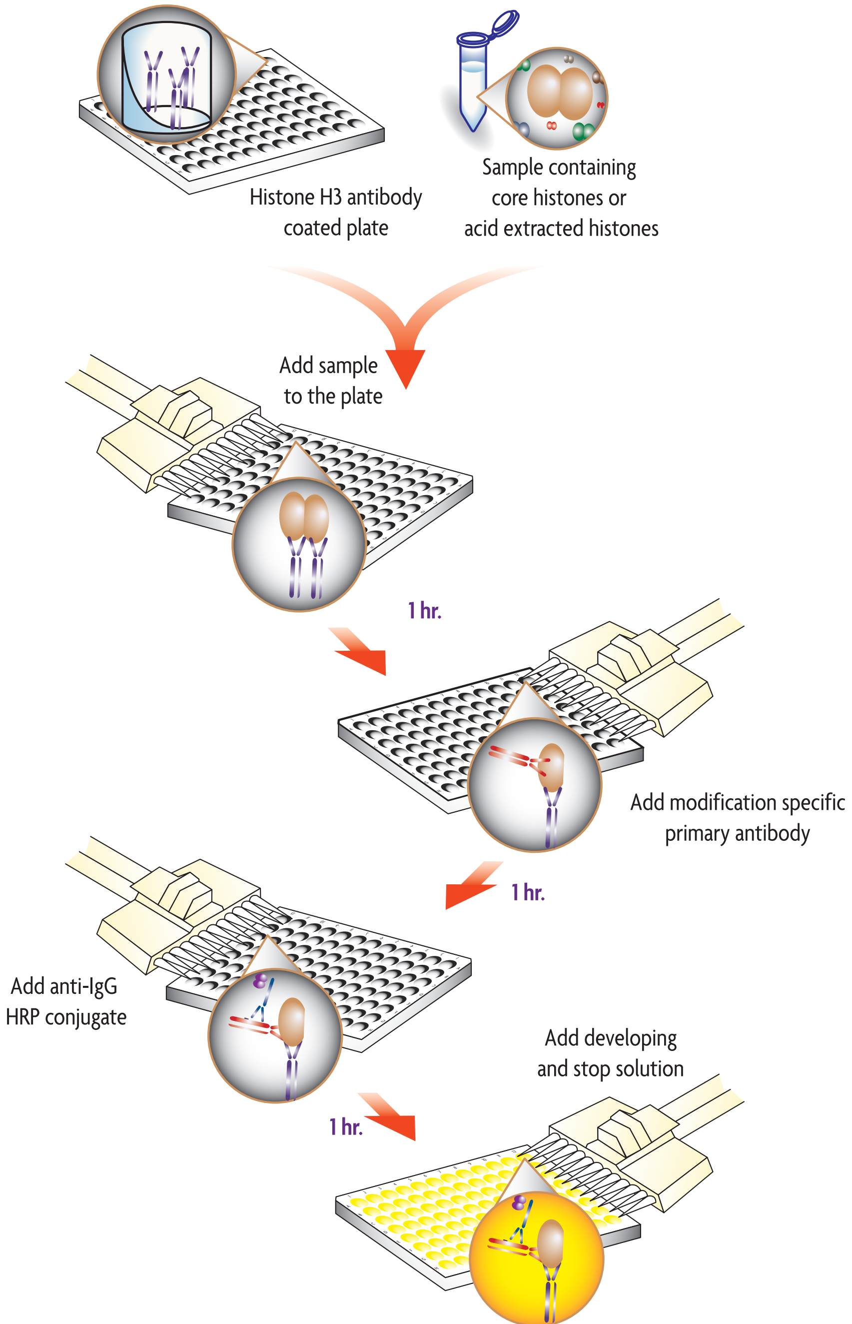
The addition or removal of modifications such as phospho-, methyl- and acetyl- functional groups to histones can have a profound effect on nuclear signaling as these dynamic modifications are critical in the regulation of transcription, chromosome packaging, DNA damage repair and functional genomics. 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 acetylated Lys27 ELISA is a simple solution for screening levels of acetylated lysine 27 (K27ac) on histone H3 in human, primate, 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 mouse monoclonal Histone H3 acetyl Lys27 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 acetylated histone H3 on lysine 27 within a range of 125 nanograms to 2 micrograms of core histone preparations and 2.5 to 20 micrograms of acid extract.

For added convenience and a more quantitative interpretation of results, the histone acetylation ELISA kits all include Active Motif's recombinant acetylated histone technology. Each acetylated histone ELISA kit is supplied with the a 99% pure Histone H3 recombinant protein that has been specifically acetylated at the desired lysine site. The included Recombinant Histone H3 acetyl Lys27 protein enables you to build a reference standard curve to quantitate the amount of specifically acetylated H3 Lys27 in your samples.

product	format	catalog no.
Histone H3 acetyl Lys27 ELISA	1 x 96 rxns	53116

Flow Chart of Process



Introduction

Histone H3 acetylated Lys27

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].

Histone acetylation is catalyzed by histone acetyltransferases (HATs) and has been shown to be important in the regulation of transcription, replication, DNA damage repair, and chromosomal condensation[3,4,5,6]. The transcriptionally active form of chromatin, called euchromatin, exhibits higher levels of histone acetylation than the transcriptionally silent form known as heterochromatin. Acetylation of histones is believed to result in decondensation of heterochromatin into the more relaxed euchromatin structure. This decondensation increases accessibility of regulatory proteins, such as transcription factors and DNA repair elements, to the underlying DNA. Thus histone acetylation plays an important role in the active processes associated with chromatin remodeling. Acetylation on Histone H3 lysine 27 is a marker for active enhancers and active promoters[7,8].

Active Motif's Histone Modification ELISA Kits make it is easy to screen for changes in acetylation levels. Histone H3 acetyl Lys27 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 & 40027). The sensitive, specific assays are able to detect acetylated lysine 27 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 acetylation levels of histone H3 lysine residues.

Kit Performance and Benefits

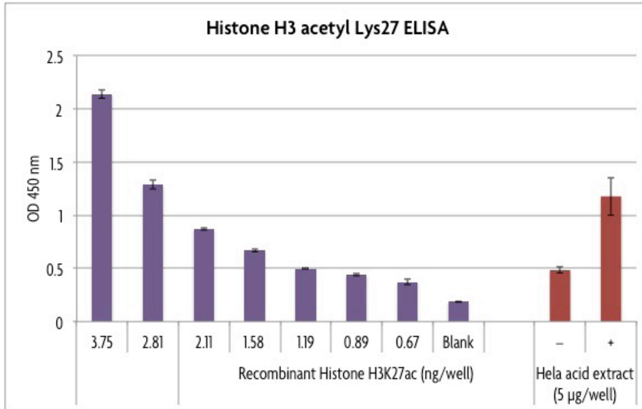
Detection limit: > 125 ng/well of purified core histones. For acid extracts, > 2.5 µg /well is recommended.

Range of detection: This ELISA provides quantitative results from 125 ng to 2 µg of purified core histones or from 2.5 µg to 20 µg of histones isolated by acid extraction. The provided Recombinant Histone H3 acetyl Lys27 protein is tested at a range of 0.67 to 3.75 ng/well to identify the linear range for a best fit curve.

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.

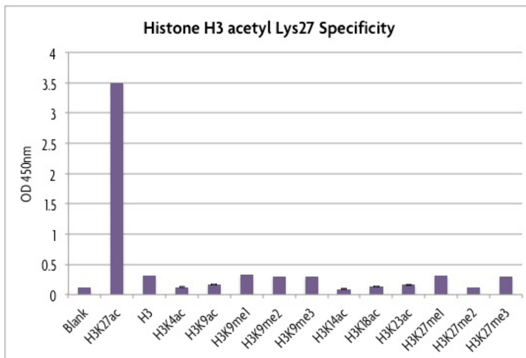
Assay time: 3.5 hours.

Histone H3 acetyl Lys27 ELISA Kit



Histone H3 acetyl Lys27 detection.

The Histone H3 acetyl Lys27 ELISA was used to assay HeLa acid extract (5 µg) either untreated (–) or treated with sodium butyrate (+), a known HDAC inhibitor, prepared as stated in Appendix Section A. The provided Recombinant Histone H3 acetyl Lys27 protein was assayed from 0.67 - 3.75 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 acetyl Lys27 specificity.

To verify the specificity of the Histone H3 acetyl Lys27 ELISA, 100 ng of Recombinant Histone H3, mono-, di- and trimethyl Lys9 and Lys27, acetyl Lys4, Lys9, Lys14, Lys18 and Lys23 proteins were assayed per well. These results show the assay specifically recognizes H3K27ac. There is extremely low background from histone H3 and little cross-reactivity with other methylated or acetylated histone modifications. This means that small, specific changes in acetyl Lys27 levels can easily be detected with this kit.

Kit Components and Storage

Histone H3 acetyl Lys27 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 acetyl Lys27 antibody	10 µl	-20°C
HRP-conjugated anti-mouse IgG1	10 µl	-20°C
Assay Dilution Buffer	18 ml	4°C
20X Wash Buffer	25 ml	4°C
Developing Solution	11 ml	4°C
Stop Solution	11 ml	4°C
Histone H3 Capture Plate	1	4°C
Recombinant Histone H3 acetyl Lys27	5 µl (20 ng/µl)	-80°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 acetyl Lys27 antibody 1:500 with Assay Dilution Buffer. Use 50 µl per well.

Dilute the HRP-conjugated anti-mouse IgG1 antibody 1:500 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 acetylated Lys27 proteins

The Recombinant Histone H3 acetyl Lys27 protein is provided as a control for quantitating the amount of acetylated histone H3 at a concentration of 20 ng/µl. There is enough recombinant protein for at least 2 standard curves. Store protein at -80°C. Avoid multiple freeze/thaw cycles by only removing the protein from the freezer when planning to run a standard curve.

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 acetyl Lys27 Ab	0.1 µl	0.9 µl	5.4 µl	10.8 µl
	Assay Dilution Buffer	51.9 µl	449 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
Secondary Antibody	HRP-conjugated anti-mouse IgG1	0.1 µl	0.9 µl	5.4 µl	10.8 µl
	Assay Dilution Buffer	51.9 µl	449 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	17.1 ml	95 ml	190 ml
	20X Wash Buffer	100 µl	0.9 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 acetyl Lys27

Use this plate set-up example to prepare a standard curve for the Histone H3 acetyl Lys27 (K27ac) ELISA in duplicate.

	H3 K27ac											
	1	2	3	4	5	6	7	8	9	10	11	12
A	3.75 ng	3.75 ng	–	–	–	–	–	–	–	–	–	–
B	2.81 ng	2.81 ng	–	–	–	–	–	–	–	–	–	–
C	2.11 ng	2.11 ng	–	–	–	–	–	–	–	–	–	–
D	1.58 ng	1.58 ng	–	–	–	–	–	–	–	–	–	–
E	1.19 ng	1.19 ng	–	–	–	–	–	–	–	–	–	–
F	0.89 ng	0.89 ng	–	–	–	–	–	–	–	–	–	–
G	0.67 ng	0.67 ng	–	–	–	–	–	–	–	–	–	–
H	0 ng	0 ng	–	–	–	–	–	–	–	–	–	–

1. Recombinant Histones are provided at a 20 ng/µl concentration. Thaw the protein on ice. Before using, vortex the tube for 10 seconds and quick spin the contents to the bottom of the tube. Store remaining protein at -80°C and avoid multiple freeze/thaw cycles.

   In a microcentrifuge tube, dilute 2 µl of Recombinant Histone H3 acetyl Lys27 into 531 µl sterile water to a final concentration of 0.075 ng/µl. Perform this dilution fresh each time.
2. Add 200 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 quarter-fold dilution of the extracts by transferring 150 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 150 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 150 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 125 ng to 2 µg

   Acid extracts: Recommended range of 2.5 to 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 acetyl Lys27 antibody 1:500 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-mouse IgG1 antibody 1:500 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 acetyl Lys27 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 acetyl Lys27 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).

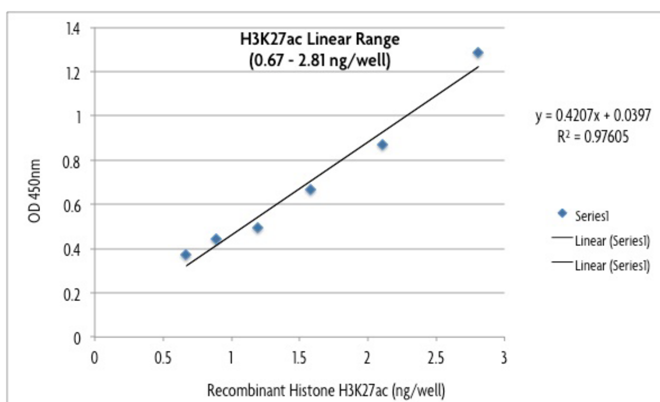
Plot the OD for the standards against the quantity (ng/well) of the standards and draw the best fit curve. The best curve fit may vary depending on the developing times used and should be calculated each time a standard curve is run. The provided Recombinant Histone acetyl Lys27 protein is tested at a range of 0.67 to 3.75 ng/well to identify the linear range for a best fit curve. To determine the concentration of modified histone in your sample, you will need to identify the linear portion of the standard curve. The linear range should be determined each time the standard curve is run. Only values that generate a linear regression with an R^2 value >0.96 should be used for analysis. The data can be linearized using log/log paper and regression analysis may also be applied.

To quantify the amount of acetyl Lys27 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 page 12.

Example curve:

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



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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 µg/well and for purified core histone, dilute down to 250 ng/well
	Concentration of antibodies is too high	Perform antibody titration to determine optimal working concentration. Start using 1:1000-1:2000 for primary antibody and 1:1000-1:2000 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 0.5 to 1 µg/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 10-20 µg/well
No signal or weak signal in standard curve wells	Too many freeze/thaw cycles of protein	Store at -80°C to avoid multiple freeze/thaws

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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