# Histone H3 PTM Multiplex Kit

Catalog No. 33115

(version B2)


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## Overview

Histone post-translational modifications (PTM) at specific amino acid residues on the N-terminal histone tails, such as phosphorylation, methylation and acetylation, can have a profound effect on cell signaling and human diseases. Many of these specific histone modifications are conserved throughout eukaryotes. Histone modifications function either by altering chromatin structure and accessibility to transcriptional machinery and/or they create binding sites for 'reader' proteins which either deposit 'write' or remove 'erase' these marks.

Active Motif's Histone H3 PTM Multiplex Kit works as a solution-based sandwich ELISA to interrogate the levels of histone modifications within acid extracted cell lysates or purified histones using either MAGPIX®, Luminex 200™ or FLEXMAP 3D® instruments. Fluorescent labeled magnetic beads, which have been conjugated to antibodies that target a specific histone PTM, are used to bind the histone within the sample. A biotinylated antibody specific for the C-terminus of Histone H3 is then added to bind the captured histone and form a 'sandwich'. Next, streptavidin-phycoerythrin (SA-PE) is introduced into the reaction to bind the biotinylated antibody. The SA-PE signal intensity is proportional to the amount of bound histone sample. Because each antibody-conjugated bead set emits a unique signal, antibodies for multiple histone modifications can be added to the same sample for multiplexing.

The Histone H3 PTM Multiplex Kit includes a 96 well assay plate, a biotinylated Histone H3 reporter antibody, streptavidin-phycoerythrin, assay buffers and an Assay Positive Control to monitor the consistency of the performance of the assay between runs. The Histone PTM antibody-conjugated beads are purchased separately. This allows the assay to be customized to fit the individual needs of each researcher. All antibody-conjugated beads have been successfully multiplexed. For normalization of PTM data across samples, the Histone H3 Total Ab-conjugated bead set is required.

**NOTE:** Bead sets MUST be purchased separately. Please refer to the specific data sheet for each Histone Antibody-conjugated Bead to identify the bead region associated with each antibody. A list of available Histone Antibody-conjugated Beads is shown on page 3, but please refer to our website at www.activemotif.com for the most up-to-date list.

| product | format | catalog no. |
|---|---|---|
| Histone H3 PTM Multiplex Kit | 96 rxns | 33115 |

This product requires the use antibody-conjugated Luminex® microspheres. Please read the Luminex terms and conditions listed at the back of this manual prior to use.

## Principle of Method

The Histone H3 PTM Multiplex Assay works as a solution-based sandwich ELISA to interrogate the levels of histone modifications within acid extracted cell lysates or purified histones. Samples are diluted in Assay Buffer and added to a 96-well assay plate. Histone post-translational modification antibodies, which are coupled to color-coded magnetic beads, are added to each well to capture histones in the sample containing the PTM of interest. Using a 96-well magnetic plate, wells are washed to remove any unbound sample. A biotinylated Histone H3 antibody is added as a reporter to recognize the C-terminal domain of the histone samples bound to the antibody-conjugated beads. Following a wash step to remove unbound biotinylated antibody, streptavidin-phycoerythrin (SA-PE) is added to each well to bind the biotinylated reporter antibody. A final wash is performed to remove unbound SA-PE and the beads are resuspended in Wash Buffer and read using a MAGPIX®, Luminex 200™ or FLEXMAP 3D® instrument and xPONENT® software.

The Luminex 200™ and FLEXMAP 3D® machine use two lasers for detection. One laser is bead-specific and determines which Histone Antibody-conjugated Bead is being detected, while the other laser determines the magnitude of the SA-PE derived signal, which is proportional to the amount of bound histone sample. The MAGPIX® instrument differs in that it utilizes a magnet to collect the beads as a monolayer in the sample chamber. LEDs are used to illuminate the chamber and CCD imaging technology captures a series of images. The software analyzes the images to determine the bead identity and phycoerythrin signal. The Luminex xPONENT software program will provide a real-time readout of signal measured as median fluorescent intensity (MFI).

Due to the availability of multiple color coded magnetic beads, each coated with its own histone modification-specific antibody, this system allows for the addition of multiple Histone Antibody-conjugated Beads to each sample for multiplexing. Please refer to the specific data sheet for each Histone Antibody-conjugated Bead to identify the associated bead region.

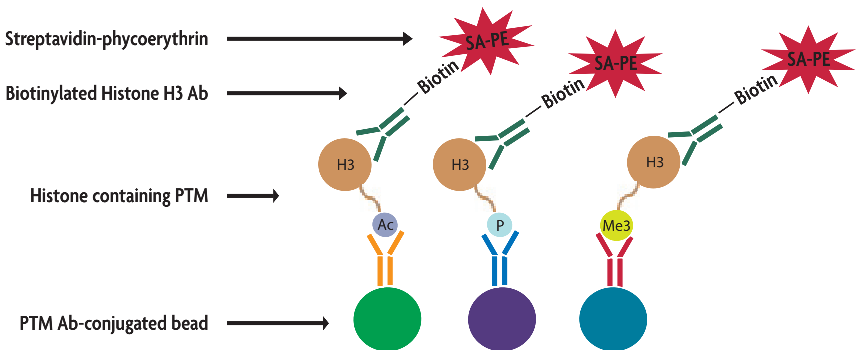


**Figure 1: Schematic of the Histone PTM Multiplex Assay.**

## Available Histone H3 Antibody-conjugated Bead Sets

| Catalog No. | Format | Components | Quantity | Storage |
|---|---|---|---|---|
| 33116 | 48 rxns | Histone H3 Total Ab-conjugated Beads | 50 µl | 4°C |
| 33117 | 48 rxns | Histone H3K9ac Ab-conjugated Beads | 50 µl | 4°C |
| 33118 | 48 rxns | Histone H3K9me1 Ab-conjugated Beads | 50 µl | 4°C |
| 33119 | 48 rxns | Histone H3K9me2 Ab-conjugated Beads | 50 µl | 4°C |
| 33120 | 48 rxns | Histone H3K9me3 Ab-conjugated Beads | 50 µl | 4°C |
| 33121 | 48 rxns | Histone H3K4me3 Ab-conjugated Beads | 50 µl | 4°C |
| 33122 | 48 rxns | Histone H3S10ph Ab-conjugated Beads | 50 µl | 4°C |
| 33123 | 48 rxns | Histone H3 pan-acetyl Ab-conjugated Beads | 50 µl | 4°C |
| 33124 | 48 rxns | Histone H3K27me2 Ab-conjugated Beads | 50 µl | 4°C |
| 33125 | 48 rxns | Histone H3K27me3 Ab-conjugated Beads | 50 µl | 4°C |
| 33126 | 48 rxns | Histone H3K27ac Ab-conjugated Beads | 50 µl | 4°C |
| 33127 | 48 rxns | Histone H3K56ac Ab-conjugated Beads | 50 µl | 4°C |
| 33128 | 48 rxns | Histone H3T11ph Ab-conjugated Beads | 50 µl | 4°C |

### Biotinylated Histone H3 Antibody

Although **Biotinylated Histone H3 antibody** is already included as a component of the Histone H3 PTM Multiplex Kit, some histone PTM specific Antibody-conjugated Beads benefit from the use of Biotinylated Histone H3 antibody reporter at higher concentrations in order to increase signal. Please refer to the specific data sheet for each Histone H3 Antibody-conjugated Bead for information on whether the use of additional Biotinylated Histone H3 antibody reporter is recommended. If frequently assaying Antibody-conjugated Bead sets for which a higher reporter antibody concentration is recommended, additional Biotinylated Histone H3 antibody can be purchased separately.

| product | quantity | catalog no. |
|---|---|---|
| Biotinylated Histone H3 antibody | 10 µl | 61513 |

## Introduction

### Histone Overview

The basic structural unit of chromatin is the nucleosome, which consists of 147 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 N-terminal 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. These histone modifications are recognized and bound by specific proteins that are coined 'writers' 'readers' and 'erasers'[1]. Many of these proteins contain highly conserved domains which serve as the building blocks of the protein structure[2]. 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[3-8].

### Histone Acetylation

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[9-12]. 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. Bromodomains are known to bind acetylated lysine residues on histones and other proteins. There are more than 70 identified members of the bromodomain family that regulate chromatin structure and gene expression as part of histone acetyltransferases or chromatin remodeling factors[13].

### Histone Methylation

Histone methylation is a post-translational modification of histones which takes place on the side chains of both lysine (K) and arginine (R) residues. Histone methylation is a reversible process which is catalyzed by histone methyltransferase enzymes, such as PRMT1 or Suv39H and is reversed by histone demethylases, such as LSD1 or Jumanji domain-containing proteins. The gene regulation consequences of histone methylation on the transcriptional state depends on the methylated residue and the degree of methylation. Lysine residues can undergo mono-, di- or trimethylation[14-17]. Additionally, arginine dimethylation can occur in either a symmetric or asymmetric state. The chromodomain family contains more than 120 members which appear to be targeting modules for methylated lysine residues on histone H3 where they are associated with the assembly of protein complexes on chromatin. Tudor domains are known to bind methylated lysine or arginine residues on Histone H3 and H4 and are believed to be involved in RNA binding, DNA damage response and chromatin modifications.

### Histone Phosphorylation

Histone phosphorylation occurs on serine and threonine residues and influences chromosome condensation, DNA repair and apoptosis. For example, phosphorylation of serines 10 and 28 on the tail of histone H3 occurs in early mitosis when chromosome condensation is induced during S-phase[18]. Phosphorylation of serine 28 on histone H3 has recently been shown to associate with destabilized nucleosomes in transcribed chromatin, making this an interesting indicator of both mitotic activity and transcriptional activation[19]. 14-3-3 proteins are known to bind to phospho-serine and phosphothreonine motifs in a sequence-specific manner and have also been found to interact directly with chromatin and chromatin modifying enzymes.

### Epigenetics and human disease

The importance of studying histone modifications has implications for human health and disease since a strong correlation exists between specific histone modifications and human pathologies, including autoimmune, neurological, inflammatory and neoplastic disorders. The diseased state is associated with altered epigenetic profiles of DNA methylation or histone modification patterns that arise from altered expression levels or activity of chromatin modifying enzymes. These changes lead to altered expression of the genes involved in cell growth or differentiation, apoptosis, DNA repair, detoxification, inflammation, cell migration or adhesion. Numerous publications have described cancer-specific changes in histone modification levels, see Table 1 below[20-30]. Relative to DNA methylation, far less is known about the biological significances of histone PTMs, mostly due to limitations in available technologies.

| | Histone H3 | | | | | Histone H4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tumor Type | Multi-Kac | K4me1-3* | K9ac | K9me1-3* | K18ac | Multi-Kac | R3me2 | K12ac | K16ac | K20me3 |
| Breast | | ● | ● | | ● | | ● | ● | ● | ● |
| Colorectal | | | | | | | | | ● | ● |
| Esophageal | | | | | ● | | ● | | | |
| Hematological | | | | | | | | | ● | ● |
| Kidney | ● | ● | | ● | ● | ● | | | | |
| Lung | | ● | | | ● | | | | | |
| Pancreatic | | ● | | ● | ● | | | | | |
| Prostate | | ● | ●* | ● | ● | ● | | | | ● |

*Recurrence predictor in high grade prostate carcinoma.

**Table 1: Histone PTMs exhibit altered levels in neoplastic tissue.**

Table 1 shows the correlation between global histone modification profiles and the overall survival prognosis for specific tumor types (Table 1 was assembled using information from References 20-30).

## Traditional Methods to Study Histone PTMs

Current methods to study histone PTMs include Western blot, immunohistochemistry and genome-wide mapping. All of these methods are time-consuming and lack high throughput capabilities. Active Motif's Histone Modification ELISAs offer one solution to the issue of time and throughput, but are limited to the analysis of a single histone PTM per ELISA. To solve these problems, Active Motif has partnered with Luminex®, the industry leader in multiplexing technology, to develop the first multiplex epigenetic assay for the study of histone PTMs.

Active Motif's Histone H3 PTM Multiplex Assay enables high throughput processing using low sample amounts (nanogram quantities) to interrogate single or multiple histone modifications within the same sample. The Histone H3 PTM Multiplex Kit includes contains a 96-well assay plate, buffers, Assay Positive Control, Biotinylated Histone H3 Antibody and streptavidin-phycoerythrin for detection. Histone modification-specific antibody-conjugated beads are used to capture the histone of interest. Since each histone antibody is conjugated to a different colored bead, multiplexing multiple histone H3 bead sets together to determine the distinct levels of each histone modification within the same sample is possible. Each PTM-specific bead set is packaged individually to allow customization of analyte selection for singleplex or multiplex analysis. Simply use the bead sets of interest in combination with the Histone H3 PTM Multiplex Kit and you will have all the reagents needed to perform the assay.

The Histone H3 PTM Multiplex Kit also offers the ability to normalize Histone H3 levels across different sample preparations. By normalizing for total H3 levels, it is possible to look at relative changes in histone PTMs across different samples. In order to perform this normalization, it is necessary to include the Histone H3 Total Antibody-conjugated Beads in combination with the Histone PTM Antibody-conjugated Beads for each sample well being tested.

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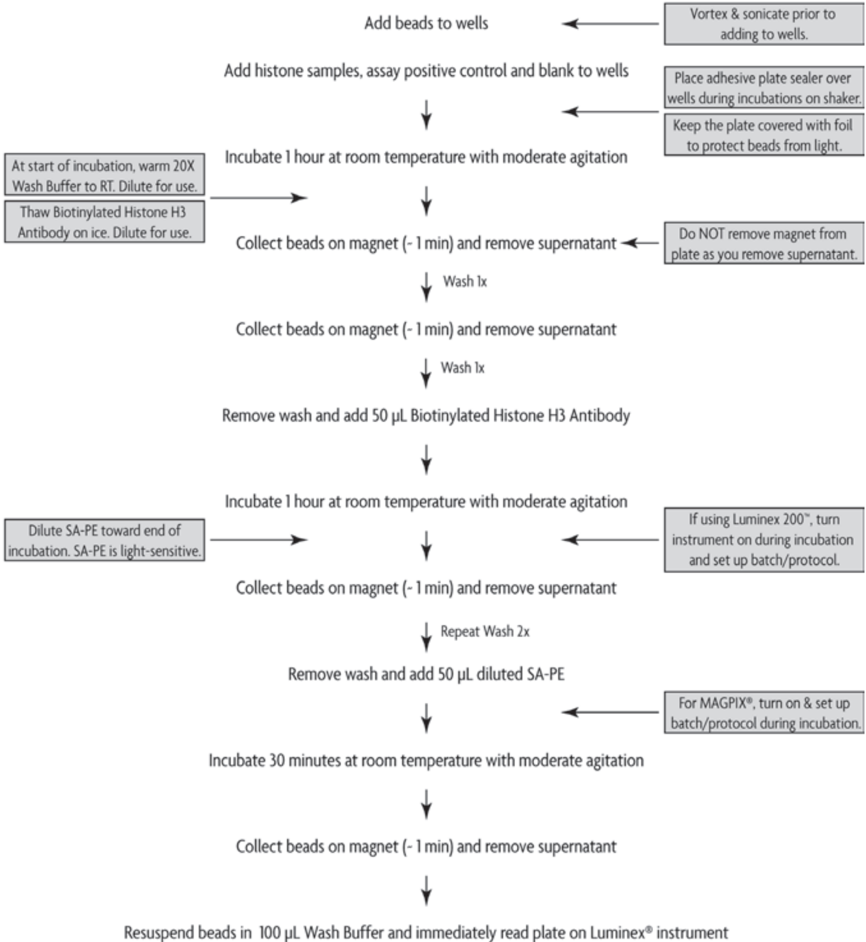
## Flow Chart of Process



## Kit Performance and Benefits

**Histone H3 PTM Multiplex Advantages:**

- First commercially available kit to perform multiplex histone analysis
- Assay works on crude acid extracts or purified histones using only nanogram quantities
- Enables analysis of single or multiple histone H3 PTM levels within the same sample
- Easy-to-use assay allows for more high throughput processing of samples
- Histone H3 Total Ab-conjugated Beads enables relative levels of histone PTMs to be determined across different histone samples by normalizing for their total H3 levels

**Detection limit:** The detection limit is specific to each Histone PTM Antibody-conjugated Bead and the type of sample used. When using Active Motif's Histone Purification Kits (Catalog Nos. 40025 & 40026) to prepare samples, purified histones H2A, H2B, H3 and H4 are obtained. Therefore, less material is required for detection within the Histone H3 PTM Multiplex Kit. Acid extracted cell lysates are a crude sample material and as a result require more sample to be used within the assay. Please refer to the data sheet for each bead for specific details on the detection limits.

**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.

**Histone H3 Normalization:** The Histone H3 PTM Multiplex Kit offers the ability to normalize Histone H3 levels across different sample preparations. By normalizing for total H3 levels, it is possible to look at relative changes in histone PTMs across different samples. In order to perform this normalization, it is necessary to include the Histone H3 Total Antibody-conjugated Beads in combination with the Histone PTM Antibody-conjugated Beads for each sample well being tested. Details regarding normalization are included in the data analysis section of the manual.

**Assay time:** 3 hours.

# Histone H3 PTM Multiplex Kit

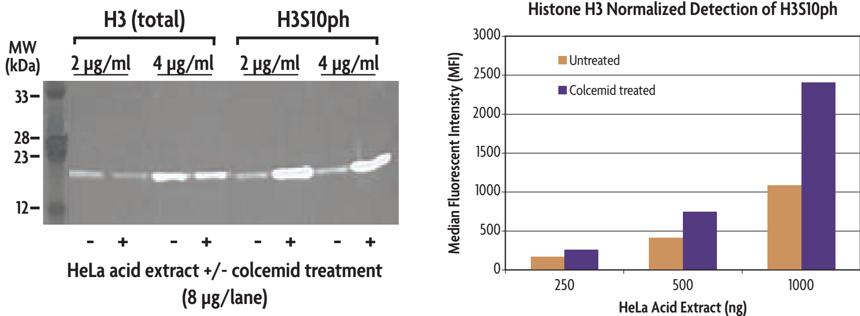



**Figure 2: Comparison of data obtained from a Western blot or Histone H3 PTM Assay for amounts of H3S10ph.**
To evaluate changes in total histone H3 and H3S10ph levels from untreated or colcemid treated Hela acid extract, a Western blot (left image) was compared with a 2-plex Histone H3 PTM assay using Total H3 and H3S10ph beads (right image). The Western blot lacks throughput capabilities, requires microgram sample quantities to study each antibody and does not provide normalized information. The Histone H3 PTM assay requires only nanogram quantities of sample from which multiple antibodies can be evaluated simultaneously in a single well. Data can then be normalized against the total H3 values to demonstrate the relative amounts of H3S10ph in each sample.

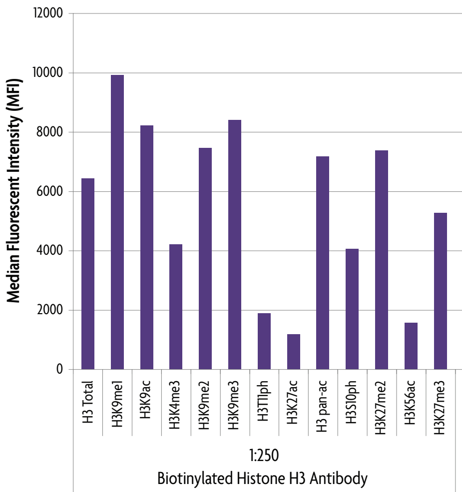



**Figure 3: Histone H3 PTM Multiplex kit using 13 Histone Antibody-conjugated Beads in multiplex.**
13 Histone Antibody-conjugated Beads were tested in multiplex on 0.5 µg Hela acid extract. Results show MFI values generated using 1:250 Biotinylated Histone H3 reporter antibody for detection. Raw MFI values are displayed as the average of duplicate wells. The combination of all 13 bead sets within a single well had no discernible effects on the assay.

## Kit Components and Storage

Histone H3 PTM Multiplex Kits are for research use only. Not for use in diagnostic procedures. Components are guaranteed for 6 months from date of receipt when stored properly.

| Reagents | Quantity | Storage |
| --- | --- | --- |
| Streptavidin Phycoerythrin (Streptavidin-PE) | 50 µl | 4°C |
| Assay Buffer AM3 | 25 ml | 4°C |
| 20X Wash Buffer | 25 ml | 4°C |
| Biotinylated Histone H3 antibody | 10 µl | -20°C |
| Deacetylase Inhibitors | 100 µl | -20°C |
| Protease Inhibitor Cocktail | 100 µl | -20°C |
| Phosphatase Inhibitor Cocktail | 100 µl | -20°C |
| Assay Positive Control | 10 µl | -80°C |
| 96-well Assay Plate | 1 ea | RT |
| Plate sealer | 2 ea | RT |

**Additional materials required**

- You MUST purchase one of Active Motif's Histone H3 Antibody-conjugated Beads to complete the assay. Each Histone PTM Antibody-conjugated Bead set provides enough beads for 48 reactions. For a list of available Histone Antibody-conjugated Bead sets, please refer to page 3 or our website www.activemotif.com.
- Histone samples (acid extracted according to Appendix G on page 23 or purified using Active Motif's Histone Purification Kits, Catalog Nos. 40025 & 40026)
- 96-well plate magnet (*e.g*. LifeSep 96F magnetic separation unit, Cat# 2501008)
- Aluminum foil
- Multi-channel pipettor and filter tips
- Multi-channel pipettor reservoirs
- Orbital shaker
- Vortex
- Mini-sonication bath (*e.g*. Cole Parmer Ultrasonic cleaner, Cat# 08849-00 or equivalent)
- Luminex® MAGPIX®, Luminex 200™ or FLEXMAP 3D® machine capable of reading Luminex bead fluorescence with xPONENT® software by Luminex Corporation

Luminex 200™, MAGPIX®, FLEXMAP 3D® and xPONENT® are registered trademarks or trademarks of Luminex Corporation.

## Reagents from Histone H3 PTM Multiplex Kit

**Preparing Histone Samples**

Histone samples can be prepared using multiple techniques:

- A simple acid extraction (see Appendix Section G) 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. More sample will be required with this method for detection within the Histone H3 PTM Multiplex Kit.
- 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. As a result of the sample purity, less sample material is required with this method for detection within the Histone H3 PTM Multiplex Kit.
- The Histone H3 PTM Multiplex Assay is optimized for detection of native histones and is NOT suitable for use with recombinant proteins that have been engineered or synthesized using *in vitro* expression systems.

Regardless of the histone preparation technique, it is recommended initially to use a range of sample concentrations (*e.g.* 50 ng, 100 ng, 200 ng, 400 ng and 800 ng) in order to determine the amount of sample necessary to fall within the detection limits of the assay. Detection limits may vary based on the Histone Antibody-conjugated Bead used, so please refer to the data sheet for the bead(s) to be tested for guidelines on sample amounts.

Histone samples should be diluted in Assay Buffer AM3 supplemented with deacetylase, protease and phosphatase inhibitors to a final volume of 25 µl per well. The amount of sample added should not exceed half the total volume (12.5 µl sample + 12.5 µl assay buffer) to avoid salt effects that can be introduced by the histone sample buffer from interfering with the assay. Samples should be assayed in duplicate.

**Assay Buffer AM3**

Assay Buffer AM3 is provided as a 1X solution. Determine how much Assay Buffer AM3 is needed (including dilution of reagents, roughly estimate 150 µl will be needed per well). Bring the buffer up to room temperature. According to the recommendations in the Quick Chart for Preparing Buffers on page 14, determine the amount of Assay Buffer AM3 needed for the dilution of the histone samples, Assay Positive Control and the Histone Antibody-conjugated Bead master mix and supplement the buffer just prior to use with deacetylase, protease and phosphatase inhibitors. These inhibitors will help to preserve the post-translational modifications within the histone samples during the initial incubation step. Subsequent wash steps will remove the enzymes that could modify the PTMs and therefore the inhibitors do not need to be included in additional reagent preparations.

**Biotinylated Histone H3 antibody**

Please refer to the specific data sheet for each Histone Antibody-conjugated Bead for information on whether the use of additional Biotinylated Histone H3 antibody is recommended. Dilute the Biotinylated Histone H3 antibody 1:500 or 1:250, as recommended on the data sheet, in Assay Buffer AM3 according to the recommendations in the Quick Chart for Preparing Buffers on page 14. Use 50 µl per well. The Biotinylated Histone H3 antibody can be kept at 4°C for up to one month.

**Streptavidin-PE**

Make a 1:100 dilution of the Streptavidin-PE in Assay Buffer AM3 according to the recommendations in the Quick Chart for Preparing Buffers on page 14. Use 50 µl per well. The Streptavidin-PE should not be frozen, but kept at 4°C.

**20X Wash Buffer**

Bring the 20X Wash Buffer up to room temperature prior to use in the assay. Prepare the amount of 1X Wash Buffer required for the assay using the recommendations in the Quick Chart for Preparing Buffers on page 14. 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. Multi-channel pipettor reservoirs may be used for dispensing the Wash Buffer into the wells being used.

**(Optional) Assay Positive Control**

The Assay Positive Control is used to monitor the consistency of the performance of the assay between runs. Inclusion of the Assay Positive Control is optional. Enough Assay Positive Control is provided with each Histone H3 PTM Multiplex Kit for 8 wells. The control is universal for all Antibody-conjugated Bead Histone H3 PTM targets. The Assay Positive Control should be assayed in separate wells in the same manner as the histone samples. Store the Assay Positive Control at -80°C.

To prepare Assay Positive Control for analysis, thaw Assay Positive Control on ice and dilute 1:25 in Assay Buffer AM3 supplemented with deacetylase, protease and phosphatase inhibitors (to make enough for two wells, dilute 2.5 µl of control into 57.5 µl of Assay Buffer AM3 supplemented with inhibitors). Use 25 µl of diluted control per well.

**(Optional) Reference Histone Curve**

The Histone H3 PTM Multiplex Kit is not a quantitative assay. However, a reference histone sample can be used to create calibration curves for relative quantification of different PTM levels. It is important to determine the concentration of the reference sample. We recommend using histones purified with Active Motif's Histone Purification Kit (Catalog Nos. 40025 & 40026) to prepare the reference sample due to the high purity obtained from this method. The Histone H3 PTM Multiplex Assay is optimized for detection of native histones and is NOT suitable for use with recombinant proteins that have been engineered or synthesized using *in vitro* expression systems. A two-fold serial dilution of the reference sample should be performed across the dynamic range of the assay. Run these samples in the assay according to the protocol. These reference samples can be analyzed within the xPONENT software using the five parameter logistic curve fitting model.

## Quick Chart for Preparing Buffers*

| Reagents to prepare | Components | 1 well | 8 wells | 16 wells | 24 wells | 48 wells | 96 wells |
|---|---|---|---|---|---|---|---|
| Assay Buffer AM3 with Inhibitors | Assay Buffer AM3 | 48.5 µl | 485 µl | 970 µl | 1.36 ml | 2.62 ml | 5.24 ml |
| | Deacetylase Inhibitor | 0.5 µl | 5 µl | 10 µl | 14 µl | 27 µl | 54 µl |
| | Protease Inhibitors | 0.5 µl | 5 µl | 10 µl | 14 µl | 27 µl | 54 µl |
| | Phosphatase Inhibitors | 0.5 µl | 5 µl | 10 µl | 14 µl | 27 µl | 54 µl |
| | **TOTAL REQUIRED** | **50 µl** | **500 µl** | **1 ml** | **1.4 ml** | **2.7 ml** | **5.4 ml** |
| Biotinylated Antibody (1:500) | Biotinylated H3 Ab | 0.1 µl | 0.9 µl | 1.7 µl | 2.6 µl | 5 µl | 10 µl |
| | Assay Buffer AM3 | 49.9 µl | 449 µl | 848 µl | 1.3 ml | 2.5 ml | 5 ml |
| | **TOTAL REQUIRED** | **50 µl** | **450 µl** | **850 µl** | **1.3 ml** | **2.5 ml** | **5 ml** |
| Biotinylated Antibody (1:250) | Biotinylated H3 Ab | 0.2 µl | 1.8 µl | 3.4 µl | 5.2 µl | 10 µl | 20 µl |
| | Assay Buffer AM3 | 49.8 µl | 448 µl | 846 µl | 1.3 ml | 2.5 ml | 5 ml |
| | **TOTAL REQUIRED** | **50 µl** | **450 µl** | **850 µl** | **1.3 ml** | **2.5 ml** | **5 ml** |
| Streptavidin-PE | Streptavidin-PE | 0.5 µl | 4.5 µl | 8.5 µl | 13 µl | 25 µl | 50 µl |
| | Assay Buffer AM3 | 49.5 µl | 445 µl | 842 µl | 1.29 ml | 2.5 ml | 5 ml |
| | **TOTAL REQUIRED** | **50 µl** | **450 µl** | **850 µl** | **1.3 ml** | **2.5 ml** | **5 ml** |
| 1X Wash Buffer | 20X Wash Buffer | 25 µl | 250 µl | 0.5 ml | 0.75 ml | 1.5 ml | 2.75 ml |
| | Distilled water | 475 µl | 4.75 ml | 9.5 ml | 14.25 ml | 28.5 ml | 52 ml |
| | **TOTAL REQUIRED** | **500 µl** | **5 ml** | **10 ml** | **15 ml** | **30 ml** | **55 ml** |

*Volumes listed in the chart for multiple well preparations include excess for pipetting.

## Reagents from Histone Antibody-conjugated Bead Set

### Preparation of Histone Antibody-conjugated Beads

Please refer to the data sheet for modification-specific details regarding the Histone Antibody-conjugated Beads. Do not freeze the beads, the beads must be stored at 4°C. Do not mix beads for storage. Prior to use, briefly centrifuge the beads to collect any liquid trapped in the cap during shipment. Vortex the vial for 5 seconds at maximum speed. Then sonicate the vial for 20 seconds in a mini-sonication bath in order to disrupt any bead aggregates. Use a P10 to P200 pipette and tip to pipet beads from their stock tube. Dilute the beads 1:25 in Assay Buffer AM3 with Inhibitors. Follow the recommendations in the table below for singleplex or multiplex assays. Use 25 µl diluted beads per well.

**Note:** For normalization of Histone H3 levels between different samples, it is necessary to multiplex the Histone H3 Total Ab-conjugated Beads in combination with the Histone PTM Ab-conjugated Bead(s) for each sample. When multiplexing a high number of beads, it has been observed that inter-assay variability can increase when beads are multiplexed. If this occurs and inter-assay normalization is required, it is recommended to run a lower plex (less than 8 multiplexed beads) assay that includes Histone H3 Total beads and the beads of the histone PTMs of interest to confirm relative histone PTM levels.

**Singleplex Bead Master Mix***

| | 1 well | 8 wells | 16 wells | 24 wells | 48 wells | 96 wells |
|---|---|---|---|---|---|---|
| Histone Ab-conjugated Bead | 1 µl | 9 µl | 17 µl | 26 µl | 50 µl | 100 µl |
| Assay Buffer AM3 with Inhibitors | 24 µl | 216 µl | 408 µl | 624 µl | 1.2 ml | 2.4 ml |
| **TOTAL REQUIRED** | **25 µl** | **225 µl** | **425 µl** | **650 µl** | **1.25 ml** | **2.5 ml** |

**Multiplex Bead Master Mix***

| | 1 well | 8 wells | 16 wells | 24 wells | 48 wells | 96 wells |
|---|---|---|---|---|---|---|
| Volume to add of each Histone Ab-conjugated Bead | 1 µl ea | 9 µl ea | 17 µl ea | 26 µl ea | 50 µl ea | 100 µl ea |
| Assay Buffer AM3 with Inhib. (n = number of bead sets) | 25 µl - (n x 1 µl) | 225 µl - (n x 9 µl) | 425 µl - (n x 17 µl) | 650 µl - (n x 26 µl) | 1.25 ml - (n x 50 µl) | 2.5 ml - (n x 100 µl) |
| **TOTAL REQUIRED** | **25 µl** | **225 µl** | **425 µl** | **650 µl** | **1.25 ml** | **2.5 ml** |

* Volumes listed in the chart for multiple well preparations include excess for pipetting.

## Recommendations

The Histone Antibody-conjugated Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with aluminum foil during all incubation steps

Do not freeze Histone Antibody-conjugated Beads, the beads must be stored at 4°C.

The orbital shaker should be set at a speed to provide maximum mixing without splashing the liquid contents outside of the wells and onto the adhesive plate sealer.

Follow the instructions and recommendations of your Luminex instrument for proper plate reading and software analysis.

For some Ab-conjugated beads, it is recommended that higher concentrations of Biotinylated Histone H3 antibody be used to increase the reporter signal. Please refer to the data sheet of the individual Histone H3 Antibody-conjugated Beads for modification-specific suggested guidelines for dilution of the Biotinylated Histone H3 antibody.

The assay should be performed under ambient temperatures (20-24°C). Performing the assay at temperatures outside this range may lead to fluctuations in Median Fluorescent Intensity (MFI) values.

Bring Assay Buffer AM3 and 20X Wash Buffer up to room temperature prior to use in the assay.

Use a P10 to P200 pipette and tip to pipet beads from the Histone Antibody-conjugated Bead vial(s) to minimize loss of beads. Do not use a 1 ml bore pipet tip to pipet beads.

We recommend the use of a repeat pipettor for dispensing the Histone Antibody-conjugated Bead master mix, Biotinylated Histone H3 antibody and the Streptavidin-PE solutions. However, these items can also be manually added.

Multi-channel pipettors can be used to perform all Wash steps

When multiplexing a high number of beads, it has been observed that increased inter-assay variability can result. If this occurs and inter-assay normalization is required, it is recommended to run a lower plex (less than 8 multiplexed beads) assay that includes Histone H3 Total beads and the beads of the histone PTMs of interest to confirm relative histone PTM levels..

## Assay Protocol

### Read the entire protocol before use.

**Note:** Bring Assay Buffer AM3 and 20X Wash Buffer up to room temperature before use in the assay.

### Section A: Plate Setup

Determine the number of wells required for testing samples, diluted Assay Positive Control and blanks in duplicate. The diluted Assay Positive Control should be assayed in separate wells in the same manner as the histone samples using the Histone Antibody-conjugated Beads specific for the histone PTM target being analyzed for detection. Place a portion of the adhesive plate sealer over any wells not to be used in the experiment. Wells that are kept dry and protected can be used for future experiments. An example plate layout is shown below.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Blank | Blank | – | – | – | – | – | – | – | – | – | – |
| B | Sample 1 | Sample 1 | – | – | – | – | – | – | – | – | – | – |
| C | Sample 2 | Sample 2 | – | – | – | – | – | – | – | – | – | – |
| D | Sample 3 | Sample 3 | – | – | – | – | – | – | – | – | – | – |
| E | Sample 4 | Sample 4 | – | – | – | – | – | – | – | – | – | – |
| F | Sample 5 | Sample 5 | – | – | – | – | – | – | – | – | – | – |
| G | Sample 6 | Sample 6 | – | – | – | – | – | – | – | – | – | – |
| H | Pos Ctl | Pos Ctl | – | – | – | – | – | – | – | – | – | – |

**Note:** To help with plate set-up, a downloadable blank 96-well plate template is available on our website at www.activemotif.com/96grid.

### Section B: Addition of Histone Antibody-conj. Beads and Sample

It is recommended to try a range of sample concentrations in order to determine the amount of sample necessary to fall within the detection limits of the assay (*e.g.* 50 ng, 100 ng, 200 ng, 400 ng and 800 ng). Please refer to the data sheet for each bead set to determine the detection limits of the Histone Antibody-conjugated Beads being used.

Histone Antibody-conjugated Beads are light sensitive. To prevent photobleaching, exposure to light should be minimized by covering the plate with aluminum foil during all incubation steps.

1. Thaw histone samples and Assay Positive Control (optional) on ice.

2. Prepare singleplex or multiplex bead master mix as described in the Buffer Preparation and Recommendation section on page 15. Vortex beads before adding to the wells. Add 25 µl of Histone Antibody-conjugated Bead master mix to each well, including Assay Positive Control and blank wells.

3. Dilute Assay Positive Control and histone samples as needed in Assay Buffer AM3 with Inhibitors to a final volume of 25 µl per well according to the Buffer Preparation and Recommendations and Quick Chart for Preparing Buffers sections on pages 13 and 14, respectively.

| | |
|---|---|
| **Assay Positive Control wells** | Add 25 µl of diluted Assay Positive Control per well. |
| **Blank wells** | Add 25 µl Assay Buffer AM3 with Inhibitors per well. |
| **Sample wells** | Add 25 µl of diluted histone samples per well. |

4. Place the adhesive plate sealer over the wells, then cover with aluminum foil to protect the beads from light. Incubate for 1 hour at room temperature on an orbital plate shaker set to slow to moderate agitation such that no sample splashes onto the adhesive sealer.

   **Note:** In preparation for use in Section C, warm 20X Wash Buffer to room temperature at the start of the incubation and dilute to 1X according to the recommendations in the Quick Chart for Preparing Buffers on page 14. Also, toward the end of the incubation, thaw Biotinylated Histone H3 Antibody on ice.

## Section C: Binding of Biotinylated Histone H3 Antibody

1. Remove aluminum foil and adhesive plate sealer from the plate. Then, place the plate on a 96-well magnet for 1 minute while keeping the aluminum foil over the plate to prevent photobleaching of the beads. During this incubation, dilute the Biotinylated Histone H3 antibody 1:500 or 1:250, as recommended on the specific data sheet for each Histone Antibody-conjugated Bead, into Assay Buffer AM3 according to the recommendations in the Quick Chart for Preparing Buffers on page 14.
2. Remove the aluminum foil. While the assay plate is still on the magnet, remove the supernatant from the wells into a liquid waste receptacle by quickly inverting the plate. Immediately blot the plate/magnet assembly on an absorbent towel to remove any excess liquid.
3. Remove the assay plate from the magnet and add 100 µl 1X Wash Buffer to the wells. Agitate the plate by hand for 10-15 seconds. Return the plate to the magnet and cover with aluminum foil for 1 minute.
4. Repeat steps 2-3 for a total of two washes. Remove the plate from the magnet after the last wash has been discarded.
5. Add 50 µl per well of the diluted Biotinylated Histone H3 antibody to all wells containing beads.
6. Cover the plate with plate sealer, then cover with aluminum foil to protect the beads from light. Incubate for 1 hour at room temperature on an orbital plate shaker set to slow to moderate agitation.

## Section D: Binding of Streptavidin-PE

1. Remove aluminum foil and adhesive plate sealer from the plate. Then, place the plate on a 96-well magnet for 1 minute while keeping the aluminum foil over the plate to prevent photobleaching of the beads. During this incubation, prepare the 1:100 dilution of Streptavidin-PE into Assay Buffer AM3.
2. Remove the aluminum foil. While the assay plate is still on the magnet, remove the supernatant from the wells into a liquid waste receptacle by quickly inverting the plate. Immediately blot the plate/magnet assembly on an absorbent towel to remove any excess liquid.
3. Remove the assay plate from the magnet and add 100 µl 1X Wash Buffer to the wells. Agitate the plate by hand for 10-15 seconds. Return the plate to the magnet and cover with aluminum foil for 1 minute.
4. Repeat steps 2-3 for a total of two washes. Remove the plate from the magnet after the last wash has been discarded.
5. Add 50 µl per well of the diluted Streptavidin-PE.
6. Cover the plate with plate sealer, then cover with aluminum foil to protect the beads from light. Incubate for 30 minutes at room temperature on an orbital plate shaker set to slow to moderate agitation.

## Section E: Preparing Samples to Read on a Luminex® Instrument

1. Remove foil and place the plate on a 96-well magnet for 1 minute while keeping the aluminum foil over the plate to prevent photobleaching of the beads.
2. Remove the aluminum foil and confirm that the beads have collected into the bottom of the well. While the assay plate is still on the magnet, remove the supernatant from the wells into a liquid waste receptacle by quickly inverting the plate. Immediately blot the plate/magnet assembly on an absorbent towel to remove any excess liquid.
3. Remove the assay plate from the magnet and add 100 µl 1X Wash Buffer to the wells. Make sure the beads are fully resuspended.
4. Following the instructions for your Luminex® instrument and xPONENT® software program, immediately read the plate.

   **Note:** If the plate is not read within 30 minutes of bead resuspension, briefly agitate the plate for 1-2 minutes on a orbital shaker immediately prior to reading the plate. Below are some recommendations on Luminex instrument settings that were used to obtain the data for this product. Please refer to the Histone Antibody-conjugated Bead data sheet for specific information on the bead set.

| Instrument | MAGPIX® | Luminex 200™ |
|---|---|---|
| Min. Events | 50 counts per bead | 50 counts per bead |
| Sample Size | 50 µl | 50 µl |
| Gate Settings | n/a | 7,500 - 20,000 |
| Reporter Gain | n/a | Default (Low PMT) |
| Time Out | n/a | 120 seconds |
| Bead Set | see data sheet | see data sheet |

## Section F: Data Analysis

1. The MFI values for each blank, sample and Assay Positive Control can be plotted using the xPONENT software or a graphical analysis software such as Excel. We recommend using net MFI values from the CSV file for analysis. Wells designated 'blanks' should be set as background in xPONENT at the plate layout step of setting up the Batch or Protocol. Assay Positive Control wells should have MFI values that are 5-fold or greater over background (blank signal). Sample MFI values will vary based on the amount of PTM present in the sample. Alternatively, the blank values can be subtracted from the sample MFI values for each bead set tested before graphical analysis. A general recommendation is that a positive signal should be at least 2 standard deviations above background (or the blank signal).
2. The use of the Histone H3 Total Ab-conjugated bead set in multiplex with a histone PTM-specific bead set allows for the normalization of differences in Histone H3 levels between different samples or treatment conditions. This enables relative comparison of PTM levels between these samples. Normalization is achieved by determining the ratio of the MFI values for the Histone H3 Total beads in the reference sample relative to the other test samples. This ratio is referred to as the Normalization Factor. Using the Normalization Factor, the MFI values of the histone PTM beads are adjusted accordingly. See the example below.

| | H3 Total Bead MFI | H3K9ac Bead MFI | H3K9me3 Bead MFI | H3K4me3 Bead MFI |
|---|---|---|---|---|
| Sample 1 | 1000 | 2000 | 1200 | 1600 |
| Sample 2 | 1500 | 2500 | 1000 | 2000 |
| Sample 3 | 750 | 1200 | 1400 | 900 |

a. Select one of the histone samples tested to be the normalizing standard. All other histone samples will be adjusted relative to this sample. The MFI values for the normalizing standard sample will remain constant.

| | H3 Total Bead MFI | H3K9ac Bead MFI | H3K9me3 Bead MFI | H3K4me3 Bead MFI |
|---|---|---|---|---|
| **Sample 1** | **1000** | **2000** | **1200** | **1600** |
| Sample 2 | 1250 | 2500 | 1000 | 2000 |
| Sample 3 | 750 | 1200 | 1400 | 900 |

Sample 1 is set as the normalizing standard in this example.

b. Create a total H3 Normalization Factor for the other histone samples by dividing the MFI values of the Histone H3 Total beads in each sample by the MFI value of the Histone H3 Total beads in the sample that was selected to be the normalizing standard.

$$\text{Normalization Factor} = \frac{\text{H3 Total MFI of sample}}{\text{H3 Total MFI of normalizing standard}}$$

| | H3 Total Bead MFI | Normal. Factor |
|---|---|---|
| **Sample 1** | **1000** | **1** |
| Sample 2 | 1250 | 1.25 |
| Sample 3 | 750 | 0.75 |

c. To adjust the Histone PTM Bead MFI values between the different samples, divide the MFI values of each PTM bead set by the Normalization Factor. This gives MFI values that are normalized based on the amount of Histone H3 that was present in each sample.

$$\text{Normalized PTM MFI} = \frac{\text{H3 PTM MFI of sample}}{\text{Normalization Factor of sample}}$$

| | Normal. Factor | H3K9ac Bead MFI | Normal. H3K9ac MFI value | H3K9me3 Bead MFI | Normal. H3K9me3 MFI value | H3K4me3 Bead MFI | Normal. H3K4me3 MFI value |
|---|---|---|---|---|---|---|---|
| **Sample 1** | **1** | **2000** | **2000** | **1200** | **1200** | **1600** | **1600** |
| Sample 2 | 1.25 | 2500 | 2000 | 1000 | 800 | 2000 | 1600 |
| Sample 3 | 0.75 | 1200 | 1600 | 1400 | 1867 | 900 | 1200 |

d. The normalized PTM MFI values show the relative amount of each histone PTM in the different samples. These normalized PTM MFI values can be graphed for analysis.

| | **Normal. H3K9ac MFI value** | **Normal. H3K9me3 MFI value** | **Normal. H3K4me3 MFI value** |
|---|---|---|---|
| **Sample 1** | **2000** | **1200** | **1600** |
| Sample 2 | 2000 | 800 | 1600 |
| Sample 3 | 1600 | 1867 | 1200 |

## Section G. Preparation of Acid Extract/Crude Histones

This procedure can be used to prepare crude acid extracted histones from a nearly confluent cell layer on a 150 mm plate, or from a 150 $cm^2$ flask or tissue material. For HeLa cells, the yield is approximately 0.15 mg of nuclear proteins from 9 x $10^6$ cells. Adjust as needed for your cell type.

**Adherent Cells:**

1. Culture cells as appropriate. Grow cells to 80-90% confluency.
2. Treat cells as desired.
3. Discard cell growth medium and wash the cells twice with 5 ml PBS per 150 mm plate.
4. Add 5 ml PBS and scrape cells from the plate and transfer to a 50 ml conical tube.
5. Pellet the cells by centrifugation in a pre-cooled 4°C rotor at 200 x g for 5-10 minutes.
6. Aspirate as much of the PBS as possible without disturbing the cell pellet.
7. Resuspend the cell pellet in 5 volumes of ice-cold Lysis Buffer (see below). Pipet the cells up and down to homogenize them into the solution.
8. Proceed to step 9.

**Suspension Cells:**

1. Culture cells as appropriate. Grow cells to 80-90% confluency. If necessary, gently scrape the cells from the sides of the flask while keeping them in their culture medium.
2. Treat cells as desired.
3. Transfer cells with the cell growth medium to a 50 ml conical tube. Pellet the cells by centrifugation in a pre-cooled 4°C rotor at 200 x g for 5-10 minutes.
4. Discard cell growth medium and wash the cells two times with 5 ml PBS per 150 $cm^2$ flask and centrifuge at 200 x g for 5 minutes at room temperature per wash.
5. After the second wash, aspirate any remaining PBS without disturbing the cell pellet. Resuspend the cells pellet in 5 volumes, or 1.5 ml per every 8 million cells, of ice-cold Lysis Buffer (see below). Pipet the cells up and down to homogenize them into the solution.
6. Proceed to step 9.

**Tissue:**

1. Homogenize the tissue completely in an ice-cold dounce homogenizer (Active Motif, Catalog No. 40401) using as little ice-cold Lysis Buffer as possible, as this will help ensure a highly concentrated extract. Keep the homogenate on ice.
2. Transfer the homogenate into a fresh microcentrifuge tube.
3. Proceed to step 9.

**Cell Lysis:**

9. Incubate the cells in Lysis Buffer (see below) for 30 minutes on a rotating platform at 4°C.
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. Record the volume of the supernatant : ________
12. Immediately neutralize the acid extracted proteins by adding 2/5 the total volume of Neutralization Buffer supplemented with DTT, deacetylase, protease and phosphatase inhibitors immediately prior to use (see below). (For example if the supernatant volume is 250 µl, add 100 µl Neutralization Buffer for a final volume of 350 µl.) Check the pH of the stabilized solution. If it is still acidic, continue to add Neutralization Buffer until the pH reaches 8.0.
13. Immediately aliquot the crude histone acid extract in small volumes to avoid multiple freeze/thaws. Reserve an aliquot for quantifying protein concentration in step 15.
14. Store the protein at -80°C for long-term stability.
15. 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 H3 PTM Multiplex Kit. The quantity of acid extract tested in the Histone H3 PTM Multiplex Kits are based on total protein determination values.

**Lysis Buffer:**
0.4 M HCl

**Neutralization Buffer:**
1 M Sodium phosphate, dibasic (*e.g*. Sigma-Aldrich, Cat# S7907)
Adjust the pH to 12.5 with 5 M NaOH

For every 100 µl Neutralization Buffer required, add the following inhibitors immediately prior to use:
2.5 µl 100 mM DTT
3.5 µl Deacetylase Inhibitor (Active Motif, Catalog No. 37494)
3.5 µl Protease Inhibitor Cocktail (Active Motif, Catalog Nos. 37490 & 37491)
3.5 µl Phosphatase Inhibitor Cocktail (Active Motif, Catalog Nos. 37492 & 37493)

## Section H: 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. |
| | Plate reader settings not optimal | Verify the settings in the xPONENT software and confirm the bead region selected matches with the bead set used. |
| | Incorrect assay temperature, timings or agitation | Check assay conditions and follow the recommendations listed in the manual. |
| High background in all wells | Cross-well contamination | Avoid cross-well contamination by using the plate sealer to cover wells and ensure that agitation settings are not too high to cause sample to splash out of wells. If using multi-channel pipets, do not allow pipet tips to touch the reagents in the plate. |
| | Concentration of reporter antibody or SA-PE is too high | Increase the dilutions used for the Biotinylated Histone H3 reporter antibody to 1:750 and the dilution for the SA-PE to 1:150. |
| | Inadequate washing | Ensure all wells are filled with Wash Buffer and follow washing recommendations. |
| High background in sample wells | Too much sample per well | Decrease amount of sample per well. Run a titration of sample to determine the assays dynamic range. Dilute the sample to a level that falls within the dynamic range and repeat the assay. |
| Unexpected change in MFI values from previous assay | Sample was not diluted prior to use in assay | Samples must be diluted into Assay Buffer containing deacetylase, protease and phosphatase inhibitors prior to use in the assay. The sample volume should not exceed 12.5 µl, which is half of the volume used per well. This is designed to prevent salt effects from the histone sample preparation from interfering with the assay. |
| | Assay was run at a different temperature | The assay should be performed under ambient temperatures (20-24°C). Performing the assay at temperatures outside this range may lead to reduced Median Fluorescent Intensity (MFI) values. |
| No signal or weak signal in sample wells | Not enough sample per well | Increase the amount of sample per well. Run a titration of sample to determine the assays dynamic range. Use enough sample to fall within the dynamic range and repeat the assay. |
| | Not enough reporter per well | Increase the amount of Biotinylated Histone H3 antibody from a concentration of 1:500 to 1:250 per well. |
| Problems making the Neutralization Buffer | 1M Sodium Phosphate, dibasic will not solubilize | 0.5 M Sodium Phosphate, Tribasic can also be used, added at rate of 200 µl for every 500 µl crude acid extract. |
| No signal or weak signal in Assay Positive Control wells | Too many freeze/thaw cycles of Assay Positive Control | Store at -80˚C to avoid multiple freeze/thaws. |

| Problem/question | Possible cause | Recommendation |
|---|---|---|
| Insufficient bead count | Bead mix prepared inappropriately | Sonicate bead vial and vortex just prior to diluting beads for use in the assay. |
| | Plate incorrectly set on magnet | Visually inspect plate/magnet assembly to ensure that the plate is uniformly in contact with the plate magnet. |
| | Probe height not adjusted correctly | Adjust the probe height with the appropriate number of alignment discs as recommended by the manufacturer's instructions. |
| | Volume in the well is not 100 µl | Ensure 100 µl Wash Buffer is in each well during the reading. Increase sample size that the instrument draws to 75 µl. |
| Beads not in region or gate | Luminex instrument not calibrated correctly or recently | Calibrate Luminex instrument based on manufacturer's instructions at least once a week. |
| | Gate settings not adjusted correctly | Recommendations are provided for the gate setting used with the Luminex 200 instrument on page 20 of the manual. |
| | Wrong bead regions | Check the data sheet for the correct bead region for the analyte being tested. |
| | Beads were exposed to light | Keep plate and bead mix covered with aluminum foil during all incubation steps. |
| | Instrument not washed or primed | Prime the Luminex instrument to remove air bubbles and wash with sheath fluid if there is any residual alcohol or sanitizing liquid |
| Problems with Luminex Instrument or software | | Please contact the manufacturer directly for issues with your Luminex instrument or xPONENT software.<br>Luminex Corporation<br>www.luminexcorp.com<br>Call 877 785-2323 in the U.S.<br>Call +1 512-381-4397 outside of the U.S. |

# Section I. Related Products

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

| 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 |
| Histone H3 acetyl Lys14 ELISA | 1 x 96 rxns | 53115 |
| Total Histone H3 ELISA | 1 x 96 rxns | 53110 |

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

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