NEW

Luminex® Histone H3 PTM Multiplex Assays

ACTIVE MOTIF®

multiplex assay system for studying histone H3 post-translational modifications

Active Motif partnered with Luminex®, the industry leader in multiplexing, to develop the Histone H3 PTM Multiplex Assay, the first multiplex epigenetic assay for high throughput analysis of histone post-translational modifications (PTMs) for use with MAGPIX®, Luminex® 200™ or FLEXMAP 3D® instruments. This unique assay enables you to gather more information using smaller sample amounts, in less time and at a lower cost than traditional methods, such as Western blot or IF.

Histone H3 PTM Multiplex Advantages

- **Multiplex up to 13 histone modifications**
- **Uses nanogram sample amounts**
- **3 hour assay**
- **High throughput 96 well plate-based format**
- **Screen PTM levels of variable compound treatments, conditions and disease states**
- **Ability to normalize and compare values across samples**

The Histone H3 PTM Multiplex Assay

Relative to DNA methylation, far less is known about the biological significance of histone PTMs, mostly due to limitations in available technologies. Current methods to study histone PTMs such as Western blot, immunostaining and genome-wide mapping, are time-consuming, lack high throughput capability and can be costly when interrogating more than one histone PTM target. To address these limitations, Active Motif developed the **Histone H3 PTM Multiplex Assay**, a highly sensitive assay that enables high throughput simultaneous interrogation of multiple histone PTMs using only nanogram sample amounts per well.

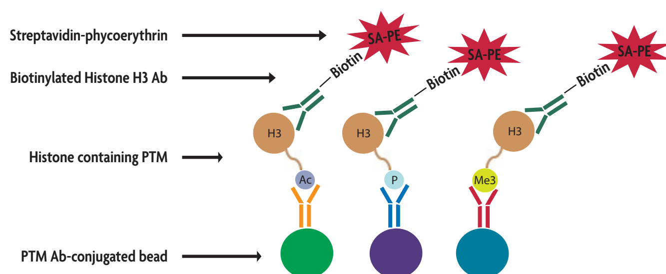

Figure 1: Schematic of the Histone H3 PTM Multiplex assay.

How Does the Assay Work?

The 96-well plate-based assay works as a solution-based sandwich ELISA to measure the levels of histone modifications in acid extracted cell lysates or purified histone samples. Histones are captured using fluorescent labeled magnetic beads that have been conjugated to antibodies specific for the histone H3 PTM target. A biotinylated antibody against the C-terminus of Histone H3 is added to bind the captured histone. Streptavidin-phycoerythrin is used to bind the biotinylated antibody and provide a readout signal to measure binding events (Figure 1).

Because the fluorescent signal is unique to each bead set, beads corresponding to multiple analytes can be multiplexed within the same sample (Figure 2). Inside the Luminex instrument, the identity of each bead is deciphered based on its emitted fluorescent signal. A second light source is applied to determine the magnitude of the streptavidin-phycoerythrin signal. The Luminex xPONENT® software program provides a real-time readout of signal as median fluorescent intensity (MFI). By including the Histone H3 Total bead set in the assay, values can be normalized to total histone H3 levels for comparison of the relative amounts of histone modifications across different samples types or treatment conditions.

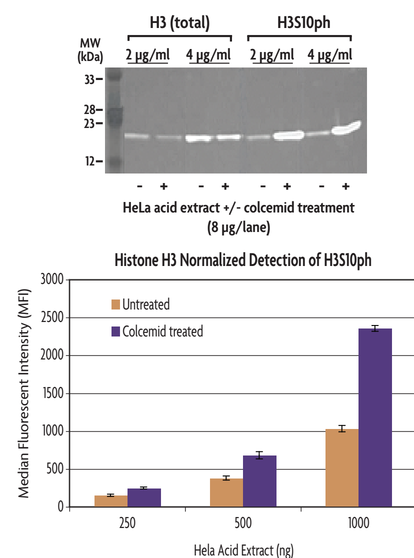

Figure 2: Comparison of H3S10ph data from a Western blot or Histone H3 PTM Assay. Total histone H3 and H3S10ph levels from untreated or colcemid-treated HeLa acid extract were evaluated by Western blot (top image) or a 2-plex Histone H3 PTM Assay using Total H3 and H3S10ph beads (bottom image). The Western is limited in throughput, requires microgram sample amounts and does not allow multiplexing or normalization of PTM values. In contrast, the Histone H3 PTM Assay enables multiplexing using only nanogram sample amounts and allows higher throughput for replicate analysis. Data normalization against total H3 values reveals relative changes in H3S10ph levels across samples.

Simultaneously Screen Specific and Off-target Effects

The Histone H3 PTM Multiplex assay enables rapid, high throughput screening of specific and off-target effects of variable conditions, compound treatments or disease states on histone modification levels. The multiplexing ability of the assay means that you can interrogate all these effects on your sample simultaneously in one well.

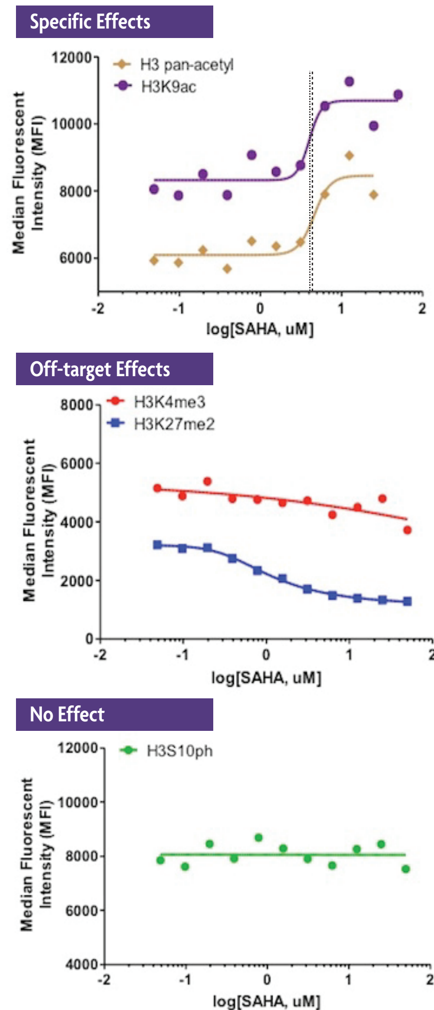

Figure 3: The Histone H3 PTM Assay shows increased histone acetylation in response to SAHA-mediated HDAC inhibition.
Two thousand HeLa cells were seeded per well and pretreated with the indicated concentrations of suberanilohydroxamic acid (SAHA), an HDAC inhibitor that is also marketed as a therapeutic compound for the treatment of cutaneous T-cell lymphoma. Acid extracts were prepared and approximately 1/10 of lysate was used to evaluate H3 pan-acetyl, H3S10ph, H3K9ac, and H3K4me3 and H3K27me2 Ab-conjugated beads in multiplex along with H3 Total beads for normalization to determine relative histone H3 PTM values using the Histone H3 PTM Multiplex Assay. The data show that the levels of H3 pan-acetyl and H3K9ac MFI signals increase in response to higher SAHA doses. IC$_{50}$ values of 4.0 µM and 4.6 µM, represented as dashed lines, were reported for H3K9ac and H3 pan-acetyl, respectively. Off-target effects and unaffected targets are also shown. Decreased levels of H3K4me3 and H3K27me2 are observed as off-target effects of SAHA treatment on histone methylation. No effect is observed on the levels of H3S10ph.

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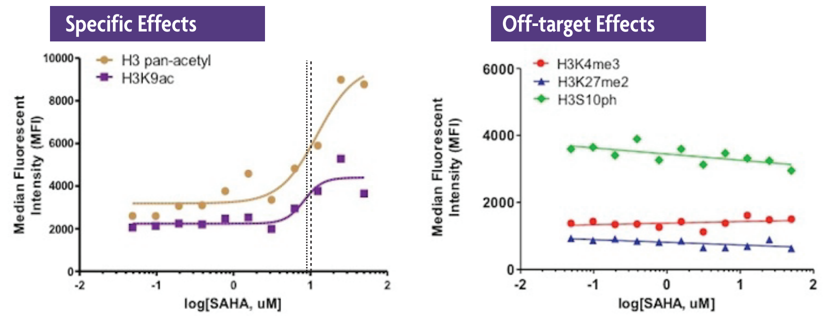

Figure 4: Histone H3 PTM Assay reveals similarities and distinctions of MCF7 cells in comparison to HeLa cells in response to SAHA treatment.
MCF7 breast cancer cells underwent the identical plating, SAHA treatment and Histone H3 PTM assay conditions as specified for HeLa cells in Figure 3. Briefly, 2000 MCF7 cells were seeded and pre-treated with SAHA at increasing concentrations. Changes in levels of histone modifications were assessed in multiplex using the Histone H3 PTM Multiplex Assay. Data reveal SAHA treatment produces similar increases in histone acetylation as were observed in HeLa cells. However, unlike HeLa cells, SAHA treatment results in an increase in H3K4me3 and a decrease in H3S10ph in MCF7 cells with little change in H3K27me2 levels. IC$_{50}$ values of 8.3 µM and 12.3 µM (dashed lines) were reported for H3K9ac and H3 pan-acetyl, respectively.

Screen Compound Effects on PTMs in Multiplex

The Histone H3 PTM Multiplex assay is ideally suited for high throughput screening of clinical samples or the effects of compounds on endogenous histone modification levels for pharma drug discovery and disease research. In the experiments shown in Figures 3 & 4, a dose response assessment was performed to determine the effect of treatment of various cell types with known histone deacetylase (HDAC) inhibitors. Figure 3 shows the effect of pre-treatment of HeLa cells with SAHA, also known as Vorinostat, that was the first HDAC inhibitor approved by the FDA for the treatment of cutaneous T cell lymphoma. Changes in the levels of acetyl, and other, H3 histone modifications produced at increasing doses of SAHA were analyzed in multiplex using the Histone H3 PTM Multiplex Assay. The results of the multiplex analysis reveal a specific and significant increase in histone acetylation in response to higher doses of SAHA, as expected, as well as non-responsive targets, such as H3S10ph. However, in addition to on-target effects and non-responders, a decrease in the levels of methyl marks (H3K4me3 and H3K27me2) are also observed as off-target effects of SAHA treatment. The assay enables you to simultaneously obtain information about specific effects, off-target effects and unaffected targets all from a single well of a 96-well plate.

The Histone H3 PTM Multiplex assay can be utilized to perform screens of multiple cell lines or model systems to assess their response to certain compounds. Figure 4 shows data obtained from SAHA-treated MCF7 breast adenocarcinoma cells under identical experimental conditions as the SAHA-treated HeLa cells shown in Figure 3. The results show similar on-target effects histone acetylation, but distinct off-target effects are observed.