

NEW: HiLite™ Binding Assays – Innovative Assay uses Fluorescence Polarization to Measure Binding of Chromatin-modifying Proteins to Histone Tails

Active Motif's new HiLite™ Binding Assay uses fluorescence polarization to provide researchers with an innovative, high-throughput tool for monitoring interactions between histone tails and chromatin-modifying proteins. The unique format of this assay enables homogenous quantitation of biologically important transcriptional regulation events, and can be used to determine the specificity of a particular protein for methylated histone tail peptides.

Identifying proteins that interact with modified histones is of great interest to the research community, as such proteins are often important regulators of genome function, transducing the histone modification to generate a specific cellular outcome. For example, HP1 and Polycomb proteins have each been shown to bind to histone H3 that has been methylated at either lysine 9 or lysine 27. Methylation of these residues is involved in specifying regions of the genome that are heterochromatic and transcriptionally silent, and both HP1 and Polycomb are known to be involved in maintaining heterochromatic regions. With Active Motif's HiLite Histone H3 Methyl-Lys9 / Lys27 Binding Assay, you can use fluorescence polarization (Figure 1) to determine if your specific protein of interest binds histone H3 that is methylated at either lysine 9 or 27. You can also measure the affinity of the binding interactions between your protein of interest and specific histone methylation states, which in turn enables fast and efficient inhibitor screening studies (Figure 2).

HiLite Binding Assay advantages

- **Homogeneous** – no washing steps required
- **Fast** – reactions reach equilibrium in just seconds to minutes
- **Reproducible** – kit reagents are stable and prepared at one time, resulting in high reproducibility
- **Robust** – resistant to changes in pH, temperature, salt concentration, etc.

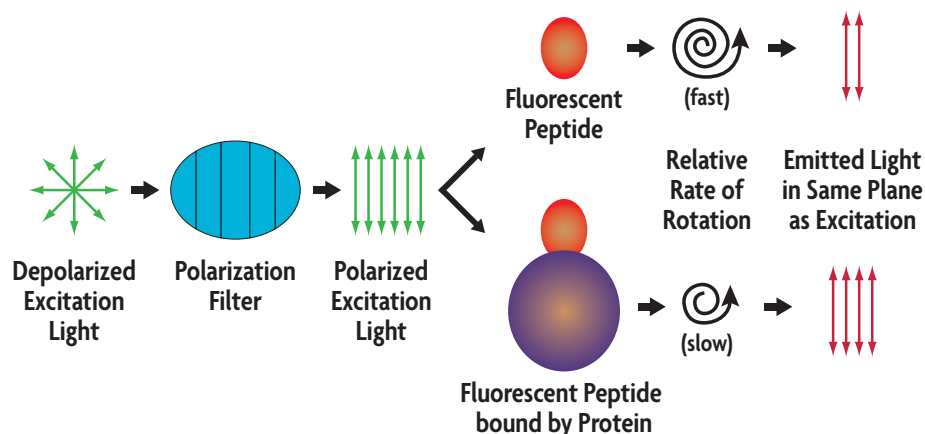


Figure 1: Schematic of the HighLite method that uses fluorescence polarization to measure protein binding.

In HiLite, various fluorescent histone peptides with site-specific modifications are added to wells containing a recombinant protein (or binding domain), then excited with polarized light. If the peptide is bound by the protein, the rotation of the "bound complex" is much slower than the rotation of unbound peptide. Slower rotation causes the amount of polarized light that is emitted in the excitation plane to be greater for the complex than for the unbound peptide. This provides a quantitative measure of the histone-binding protein's affinity for the peptide's histone modification. With the peptides included in HiLite, it is possible to determine binding equilibria for proteins that range from 10 kDa to 100 kDa.

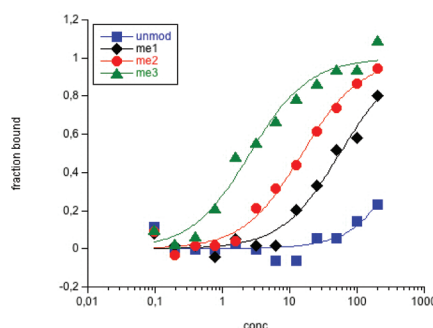


Figure 2: Binding of HP1 to histone H3 Methyl-Lys9.

One μM of fluorescent histone H3 Lys 9 peptides, which are either unmodified or mono-, di- or trimethylated at K9, were added to a 96-well plate containing a serial dilution of HP1 protein (x axis) starting at a concentration of 200 μM . The plate was scanned on a Tecan Infinite F200 using 485 nm excitation and 535 nm emission filters with polarizers. A gain of 80 was used; 100 reads were taken of each well, then averaged.

What comes in the kit?

HiLite Histone H3 Methyl-Lys9 / Lys27 contains 8 fluorescently labeled peptides that correspond to the regions of histone H3 around lysine 9 and lysine 27 that are either unmodified, or mono-, di- or trimethylated. It also contains a positive control protein, assay buffer, calibration dye and five 96-well half area black polystyrene plates. One plate is for calibrating your microplate reader and performing a binding curve with the positive control protein; the remaining four plates are to assay your protein(s) of interest. For additional details, please visit us at www.activemotif.com/hilite.

| Product | Format | Catalog No. |
|--|--------|-------------|
| HiLite™ Histone H3 Methyl-Lys9 / Lys27 Binding Assay | 1 kit | 57001 |