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Polyclonal Antibody to Methylated Lysine (Di- and Tri-) - Serum

Catalog No.: AP32611SU-N

Quantity: 0.1 ml

Background: Post-translational modifications of proteins play critical roles in the regulation and function of many known biological processes. Proteins can be post-translationally modified in many different ways, and a common posttranscriptional modification of Lysine involves methylation. Lysine can be methylated once, twice or three times by lysine methyltransferases. The transfer of methyl groups from S-adenosyl methionine to histones is catalyzed by enzymes known as histone methyltransferases. Histones which are methylated on certain residues can act epigenetically to repress or activate gene expression.
The transcriptional repressor SUV39H1 can encode novel enzymes which selectively methylate histone H3 at lysine 9. SUV39H1 places a methyl marker on histone H3, which is then recognized by HP1 through its chromo domain. This model may also explain the stable inheritance of the heterochromatic state. Some studies have also speculated a stimulatory role for transcription by methylated histone lyside 4 due to its presence at active transcription sites.

Host: Rabbit

Immunogen: KLH-conjugated, synthetic peptide containing the sequence (...AR[me2K]ST...) in which [me2K] corresponds to dimethyl-lysine 9 of human histone H3.

Format: **State:** Liquid Serum
Preservatives: 0.05% Sodium Azide

Applications: **Immunoblot Analysis:** A 1/5,000-1/10,000 dilution of this lot detected di/trimethylated Histone H3 in acid extracts from HeLa cells. No detection of unmodified recombinant histone H3 was observed (See **Figure 1**).
Peptide Dot Blot analysis: This antibody has been reported by an independent laboratory to detect di/trimethylated lysines in histones. Serial dilutions of various methylated peptides were spotted onto PVDF and probed with this antibody (See **Figure 2**).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Molecular Weight: 17 kDa

Specificity: Recognizes Di/Trimethylated Lysines.

Species Reactivity: **Tested:** Human.
Expected from sequence similarity: Broad.

Storage:

Store undiluted (in aliquots) at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Protocols:

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on an acid-extracted protein sample (see protocol below) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk in TBS (TBS-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with 1:5,000-1:10,000 dilution of anti-di/trimethyl-Lysine, pan, diluted in freshly prepared TBS-MLK for 2 hours with agitation at room temperature.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, 1/5000 dilution was used) in TBS-MLK for 30 minutes with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween®-20 for 10 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Acid Extraction of Proteins from HeLa Cells

1. Grow cells to 70% confluency in DMEM supplemented with 10% FBS.
2. Scrape the cells from the plate.
3. Pellet the cells by centrifugation at 200 x g for 10 minutes.
4. Decant the supernatant fraction.
5. Suspend the cells with 10-15 volumes of PBS and centrifuge at 200 x g for 10 minutes.
6. Decant supernatant fraction (PBS wash).
7. Suspend the cell pellet in 5-10 volumes of lysis buffer.
8. Add hydrochloric acid to a final concentration of 0.2M (0.2N). Use polypropylene tubes.
9. Incubate on ice for 30 minutes.
10. Centrifuge at 11,000 x g for 10 minutes at 4°C.
11. Keep the supernatant fraction, which contains the acid soluble proteins, and discard the acid-insoluble pellet.
12. Dialyze the supernatant against 200 mL 0.1M (0.1N) acetic acid, twice for 1-2 hours each.
13. Dialyze three times against 200 mL H2O for 1 hour, 3 hours, and overnight, respectively.

The protein can be quantified and lyophilized or stored at -70°C.

Lysis buffer:

10mM HEPES, pH 7.9
1.5mM MgCl2
10mM KCl
0.5mM DTT*
1.5mM PMSF*
*Add PMSF and DTT just prior to use of the buffer.

Pictures:

Figure 1. Immunoblot Analysis Acid extracts from HeLa cells (Lane 1) and recombinant Histone H3 (Lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-di/trimethyl-Lysine, pan (1/10,000). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Histone H3 (~17 kDa).

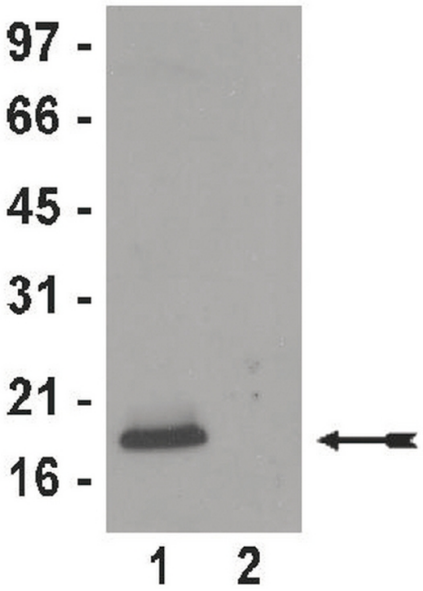

Figure 2. Peptide Dot Blot analysis: This antibody has been reported by an independent laboratory to detect di/trimethylated lysines in histones. Serial dilutions of various methylated peptides were spotted onto PVDF and probed with this antibody.