

**Certificate of Analysis**

**Anti-Histone H1 (32-33kDa)**

(mouse monoclonal IgG<sub>2a</sub>)

Catalog # 05-457

Lot # 16261

**Immunogen:** Nuclei from myeloid leukemia biopsy cells. Clone AE-4.

**Specificity:** Recognizes and is specific for histone H1 of approximately 32-33kDa. Doublet detected due to hyper-phosphorylation of histone H1 subpopulation.

**Species Cross-reactivity:** Human and bovine. Reported to stain nuclei from other animal and plant species.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

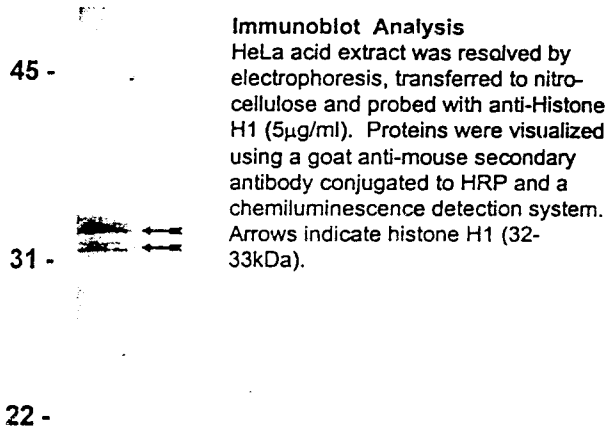
**Formulation:** 200µg of protein A purified IgG<sub>2a</sub> in 200µl of 0.1M Tris-glycine, pH 7.4, containing 0.05% sodium azide. Frozen solution.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

**Quality Control Testing**

**Immunoblot Analysis:** 1-5µg/ml of this lot detected histone H1 in lysates from an acid extract of HeLa cells.

**Immunocytochemistry:** 20µg/ml of this lot showed positive nuclear immunostaining in A431 cells fixed with 4% paraformaldehyde and methanol.



**Background:** Histones are a family of basic proteins found in all eukaryotic nuclei that are associated with eukaryotic DNA. The five major types of histone are H1, H2A, H2B, H3 and H4. The latter four, which comprise nucleosome core, are evolutionarily conserved, while the linker histone H1 exhibits greater sequence diversity among organisms. The amino acid sequence of H1 varies more from organism to organism. The separation of H1 into multiple bands by gel electrophoresis results from differences in the extent of phosphorylation of residues in the protein. This antibody can be used as a control for anti-phosphohistone H1 (Catalog # 06-597).

**General References:**

Graziano, V., *et al.*, Nature **368**: 351-354, 1994.  
Hebbes, T.R., *et al.*, EMBO J. **7**: 1395-1402, 1988.

-20°C storage  
Add A protein (mouse IgG)

#### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on an acid-extracted protein from HeLa cells (Extraction buffer: 10mM HEPES, pH7.9; 1.5mM MgCl<sub>2</sub>; 10mM KCl; 1.5mM PMSF; 0.5mM DTT and 0.4N sulfuric acid) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
1. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
2. Incubate the nitrocellulose with 1-5µg/ml of  $\alpha$ -Histone H1, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
3. Wash the nitrocellulose twice with water.
4. Incubate the nitrocellulose in the secondary reagent of choice (a goat  $\alpha$ -mouse HRP conjugated IgG, 1:3000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
5. Wash the nitrocellulose with water twice.
6. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
7. Rinse the nitrocellulose in 4-5 changes of water.
8. Use detection method of choice (enhanced chemiluminescence was used).

#### Immunocytochemistry Protocol

1. Plate approximately 200µl of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO<sub>2</sub> incubator.
2. Wash the cells three times for 5 minutes with PBS. Do not shake cells.
3. Add fix (4% paraformaldehyde) in PBS for 5 minutes at room temperature.
4. Wash the cells with PBS twice, for 15 minutes. Do not shake.
5. Add -20°C methanol for 3 minutes at room temperature.
6. Wash with PBS twice, for 10 minutes.
7. Add 400µl of 1% BSA in PBS and incubate for 1 hour at room temperature.
8. Wash the cells with PBS for 15 minutes.
9. Incubate the cells with 20µg/ml  $\alpha$ -Histone H1 in 1% BSA in PBS and incubate for 1 hour at room temperature.
10. Wash the cells twice with PBS for 5 minutes.
11. Incubate the cells with a 1:100 dilution of goat anti-mouse IgG fluorescein conjugated secondary antibody in 1% BSA in PBS for 1 hour at room temperature.
12. Wash the cells three times with PBS for 5 minutes.
13. Examine the cells under a fluorescent microscope.

#### Acid Extraction of Proteins from Sodium Butyrate Treated HeLa Cells

1. Grow cells to 70% confluency in DMEM supplemented with 10% FBS.
2. Add sodium butyrate (100mM sterile stock solution), which inhibits histone deacetylases, to a final concentration of 5mM and continue to grow the cells for 24 hours.
3. Scrape the cells from the plate.
4. Pellet the cells by centrifugation at 175 x g for 10 minutes.
5. Decant the supernatant fraction.
6. Suspend the cells with 10-15 volumes of PBS and centrifuge at 175 x g for 10 minutes.
7. Decant supernatant fraction (PBS wash).
8. Suspend the cell pellet in 5-10 volumes of lysis buffer.
9. Add sulfuric acid to a final concentration of 0.2M (0.4N). **Use polypropylene tubes.**
10. Incubate on ice for 30 minutes.
11. Centrifuge at 10,080 x g for 10 minutes at 4°C.
12. Keep the supernatant fraction which contains the acid soluble proteins and discard the acid-insoluble pellet.
13. Dialyze the supernatant against 200ml 0.1M (0.1N) acetic acid, twice for 1-2 hours each.
14. Dialyze three times against 200ml H<sub>2</sub>O for 1hour, 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 MgCl<sub>2</sub>  
10mM KCl

\*0.5mM DTT  
\*1.5mM PMSF

\*Add PMSF and DTT just prior to use of the buffer.