

Certificate of Analysis

Anti-phospho-Histone H3 (Ser10), clone 3H10

(mouse monoclonal IgG_{1κ})

Catalog # 05-806

Lot # 0609040825

Immunogen: A proprietary immunogen based on a peptide sequence containing phospho-serine corresponding to residue 10 of human histone H3. Clone 3H10

Specificity: Recognizes histone H3 phosphorylated at Ser10, Mr 17 kDa.

Species Cross-reactivity: Human. Broad species cross-reactivity is expected.

Formulation: 100 µg of protein G purified mouse IgG_{1κ} in 82 µL of 70% storage buffer (0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide) and 30% glycerol. Store at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

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

Quality Control Testing

Immunoblot Analysis: 0.05-0.5 µg/mL of this lot detected phosphorylated histone H3 in acid extracted proteins from mitotic HeLa cells treated with colcemid (Catalog # 17-306), but not unmodified recombinant Histone H3 (Catalog # 14-494) (Figure A).

Beadlyte[®] Histone-Peptide Specificity Assay: 1:1,000 to 1:81,000 dilutions of a previous lot were incubated with histone H3 peptides containing various modifications conjugated to Luminex[®] microspheres. (Figure B). Only the peptide containing phospho-serine 10 was detected.

Peptide Inhibition Analysis: Detection of histone H3 in immunoblots of colcemid treated HeLa acid extracts by anti-phospho-Histone H3 (Ser10) was diminished by 10 µM of histone H3 peptide containing phospho-serine 10, but not by peptides containing phospho-serine 28 or an unmodified histone H3 sequence (Figure C).

Immunocytochemistry: 0.2 µg/mL of a previous lot showed positive chromosome immunostaining for mitotic A431 and HeLa cells fixed with 95% ethanol and 5% acetic acid and permeabilized with 0.1% Triton[®] X-100.

Additional Research Applications

Flow Cytometry: This antibody has been reported by an independent laboratory to detect phosphorylated histone H3 using flow cytometry.

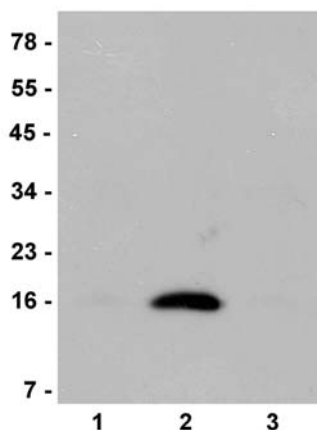


Figure A
Immunoblot Analysis
Representative blot from a previous lot. Acid extract from untreated (Lane 1) and colcemid treated (Lane 2) HeLa cells, along with recombinant Histone H3 (Lane 3) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Histone H3 (Ser10) (0.1 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phospho-histone H3 (Ser10), (~17 kDa).

General References:

Ajiro, K, *et al* (1996). Alteration of cell cycle-dependent histone phosphorylations by okadaic acid. Induction of mitosis-specific H3 phosphorylation and chromatin condensation in mammalian interphase cells. *J Biol Chem* **271**: 13197-201.

Chadee, D N, *et al* (1995). Increased phosphorylation of histone H1 in mouse fibroblasts transformed with oncogenes or constitutively active mitogen-activated protein kinase. *J Biol Chem* **270**: 20098-105.

Mahadevan, L C, *et al* (1991). Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell* **65**: 775-83.

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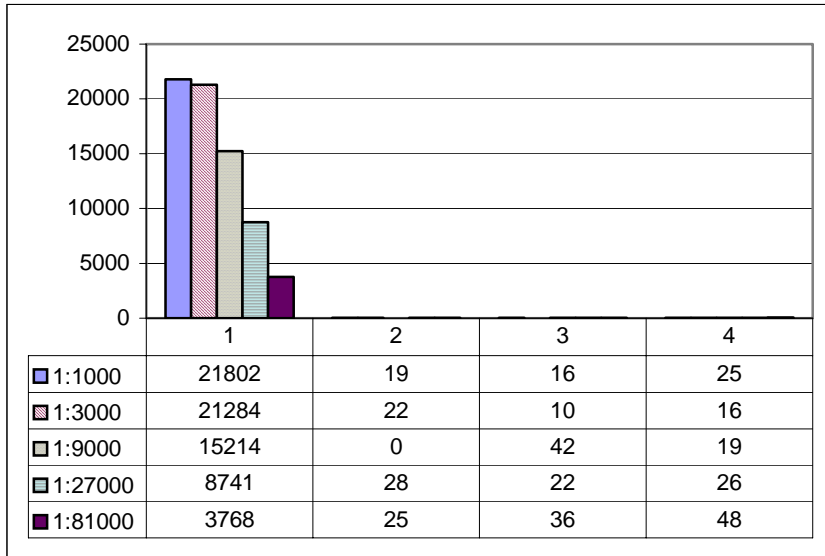


Figure B
Beadlyte® Histone-Peptide Specificity Assay
 Representative data from a previous lot. 1:1,000-1:81,000 dilutions of anti-phospho-Histone H3 (Ser10) were incubated with a cocktail of microspheres conjugated to histone H3 peptides with the following modifications:

1. phospho-serine 10
2. phospho-serine 28
3. phospho-threonine 11
4. No modifications, containing aa 1-20

Unbound antibody was removed by filtration. The peptide-antibody complexes were incubated with a biotin-conjugated anti-mouse secondary antibody followed by incubation with a phycoerythrin-streptavidin conjugate. Fluorescence was read on a Luminex® 100™ instrument. Median fluorescence intensity (MFI) is plotted.

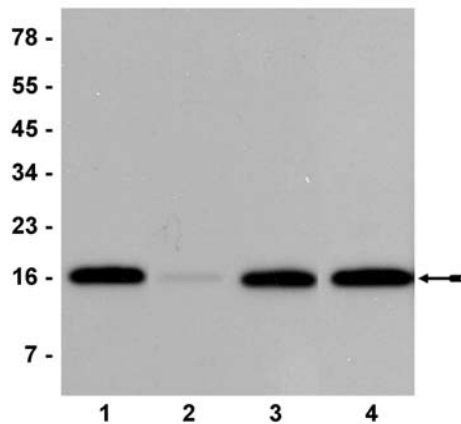


Figure C
Peptide Inhibition Analysis

Representative blot from a previous lot. HeLa acid extracts from colcemid treated cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Histone H3 (Ser10) alone (Lane 1) or the antibody pre-absorbed with 10µM of histone H3 peptides with the following modifications:

- Lane 2: phospho-serine 10
- Lane 3: phospho-serine 28
- Lane 4: no modification

A 1:10,000 dilution of primary antibody was used. Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on an acid-extracted protein sample (see protocol, page three) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200) and 0.05% Tween®-20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.05-0.5 µg/mL of anti-phospho-Histone H3 (Ser10), clone 3H10** diluted in freshly prepared TBST-MLK overnight at 4 C with agitation.
4. Wash the nitrocellulose several times with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5,000 dilution was used) in TBST-MLK for 1 hour at room temperature with agitation.
6. Wash the nitrocellulose twice with water.

7. Wash the nitrocellulose in TBS-0.05% Tween[®]-20 for 5 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. Add 50ng/ml colcemid and continue to grow the cells for 24 hours.
3. Scrape the cells from the plate.
4. Pellet the cells by centrifugation at 200 x g for 10 minutes.
5. Decant the supernatant fraction.
6. Suspend the cells with 10-15 volumes of PBS and centrifuge at 200 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 hydrochloric acid to a final concentration of 0.2 M (0.2 N). **Use polypropylene tubes.**
10. Incubate on ice for 30 minutes.
11. Centrifuge at 11,000 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 200 mL 0.1 M (0.1 N) acetic acid, twice for 1-2 hours each.
14. Dialyze three times against 200ml H₂O for 1hour, 3 hours, and overnight, respectively. The protein can be quantified and lyophilized or stored at -70°C.

Lysis buffer:

10 mM HEPES, pH 7.9
1.5 mM MgCl₂
10 mM KCl

*0.5 mM DTT
*1.5 mM PMSF

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

Immunocytochemistry Protocol

1. Plate approximately 200 μ L of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO₂ incubator at 30-40% confluency.
2. Wash the cells three times for 5 minutes with TBS. Do not shake cells.
3. Add fix (95% ethanol, 5% acetic acid) for 5 minutes at room temperature.
4. Wash the cells with TBS, three times, for 5 minutes each. Do not shake.
5. Permeabilize cells with supplemented TBS containing 0.1% Triton[®] X-100 for 5 minutes.
6. Wash the cells with TBS one time for 5 minutes. Do not shake.
7. Cover cells with 400 μ L of 1% BSA in TBS and incubate for 15 minutes at room temperature.
8. Wash the cells with TBS one time for 5 minutes.
9. Incubate the cells with **0.2 μ g/mL of anti-phospho-Histone H3 (Ser10), clone 3H10** in TBS and incubate for 1.5 hours at room temperature.
10. Wash the cells three times with TBS for 5 minutes each.
11. Incubate the cells with a 1:500 dilution of goat anti-mouse FITC secondary in TBS for 30 minutes at room temperature.
12. Wash the cells three times with TBS for 5 minutes each and once for 15 minutes.
13. Mount coverslips and examine the cells under a fluorescent microscope.