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cell signaling solutions
a Serologicals® Company

Certificate of Analysis

Anti-Phospho-Histone H3 (Ser10), clone 3H10, FITC Conjugate

(mouse monoclonal $IgG_{1\kappa}$)
Catalog # 16-222
Lot # 30436

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 17kDa.

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

Applications: Western blotting, immunofluorescence.

Formulation: **100µg** of FITC-conjugated protein G purified mouse $IgG_{1\kappa}$ in **200µl** of PBS containing 1% BSA, 0.05% Tween®-20 and 0.05% sodium azide. Frozen at -20°C.

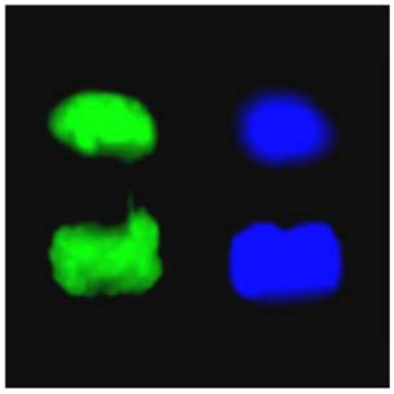
Storage and Stability: Do not store the material diluted. Stable for 1 year at -20°C from date of shipment.

Handling Recommendations: Upon first thaw, 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.**

FOR RESEARCH USE ONLY - NOT FOR USE IN HUMANS

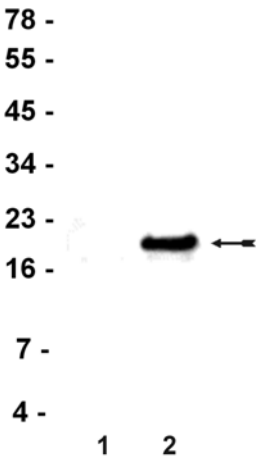
Quality Control Testing

Immunocytochemistry: Mitotic HeLa cells showed positive chromosome staining with 4µg/ml of this lot.



Immunocytochemistry
Mitotic HeLa cells were stained with 4µg/ml of this lot (green) and DAPI (blue).

Immunoblot Analysis: 0.5-5µg/ml of this lot detected phosphorylated histone H3 in acid extracted proteins from mitotic HeLa cells (Catalog # 17-306) treated with colcemid.



Immunoblot Analysis
Acid extract from untreated (Lane 1) and colcemid treated (Lane 2) HeLa cells were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Histone H3, clone 3H10, FITC Conjugate (5µg/ml). Proteins were visualized using Amersham's Typhoon 9400. Arrow indicates phospho-Histone H3 (Ser10), (~17kDa).

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose.
2. Wet the blotted nitrocellulose in PBS for 5 minutes.
3. Block the blotted nitrocellulose in Odyssey® Blocking Buffer (Li-Cor, Catalog # 927-40000) for 1 hour at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.5-5µg/ml of anti-phospho-Histone H3 (Ser10), clone 3H10, FITC Conjugate,** diluted in Odyssey® Blocking Buffer for 1 hour or longer with agitation at room temperature. Protect from light during incubation.
5. Wash the nitrocellulose 4 times for 5 minutes each at room temperature in PBS-0.05% Tween®-20 with agitation. Protect from light.
6. Rinse the nitrocellulose with PBS to remove residual Tween®-20. The membrane is now ready to scan.
7. Use detection method of choice (Li-Cor® Odyssey™ Infrared Imaging System or Amersham Biosciences Typhoon Imaging System).

Immunocytochemistry

1. Plate cells on coverslips in each well of a plate. Place the cells in a CO_2 incubator at 37ºC for 24 hours.
2. Remove media and wash the cells with PBS by rinsing twice.
3. Add fixative (3.7% formaldehyde) in PBS for 20 minutes at room temperature. Wash two times with PBS for 5 minutes.
4. Permeabilize with 0.5% Triton X-100 for 2 minutes.
5. Wash the cells twice with PBS for 5 minutes.
6. Incubate the cells with 4µg/ml of **anti-phospho-Histone H3 (Ser10), clone 3H10, FITC Conjugate** in PBS for 1 hour.
7. Wash the cells twice with PBS for 5 minutes.
8. Mount the coverslip to a slide and dry.
9. Examine the cells under a fluorescent microscope.