

## Certificate of Analysis

### Anti-Phospho-Histone H3 (Ser10), clone 3H10, Cy5 conjugate

(mouse monoclonal IgG<sub>1κ</sub>)

Catalog # 16-218

Lot # 30317

**General Description:** Protein G purified mouse IgG<sub>1κ</sub> (Catalog # 05-806), conjugated to Cy5.

**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.

**Applications:** Western blotting, immunofluorescence.

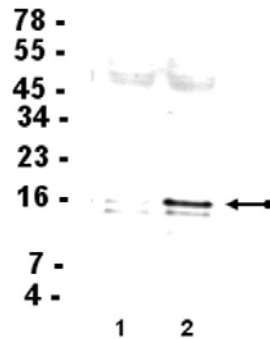
**Formulation:** 100μg Cy5-conjugated mouse IgG<sub>1κ</sub> in 200μl of PBS containing 1% BSA, 0.05% Tween<sup>®</sup>-20, 0.05% sodium azide. Liquid at 4°C.

**Storage and Stability: Do Not Freeze.** Do not store the material diluted. Stable for 1 year at 4°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing cap.

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS

### Quality Control Testing

Immunoblot Analysis: 0.2-2μg/ml of this lot detected phosphorylated histone H3 in acid extracted proteins from mitotic HeLa cells treated with colcemid (Catalog # 17-306).



#### Immunoblot Analysis

Acid extracts 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 (Ser10), Cy5 Conjugate (2μg/ml). Proteins were visualized using Amersham Typhoon<sup>™</sup> 9400 Imaging System. 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<sub>3</sub>VO<sub>4</sub>; 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<sup>®</sup> Blocking Buffer (LI-COR, Catalog # 927-40000) for 1 hour at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.2-2µg/ml of anti-phospho-Histone H3 (Ser10), clone 3H10, Cy5 conjugate**, diluted in Odyssey<sup>®</sup> 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<sup>®</sup>-20 with agitation. Protect from light.
6. Rinse the nitrocellulose with PBS to remove residual Tween<sup>®</sup>-20. The membrane is now ready to scan.
7. Use detection method of choice (Li-Cor<sup>®</sup> Odyssey<sup>™</sup> Infrared Imaging System or Amersham Biosciences Typhoon<sup>™</sup> Imaging System).

