



cell signaling solutions

## Certificate of Analysis

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### Control Histones (+/- Colcemid) (HeLa cell acid extract)

Catalog # 17-306

Lot # 24772

#### Kit Components

**Control Histones, untreated, (HeLa cell acid extract)**, Catalog # 13-112, Lot # 24773. 10 vials, each vial containing **50µg** of precipitated core histones, lyophilized from sterile, distilled water. Lyophilized powder.

**Control Histones, colcemid-treated, (HeLa cell acid extract)**, Catalog # 13-114, Lot # 24774. 10 vials, each vial containing **50µg** of precipitated core histones, lyophilized from sterile, distilled water. Lyophilized powder.

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

#### Kit Description

**Product Description:** Core histones, including histone H1, purified by acid extraction precipitation from log phase untreated and colcemid-treated HeLa cells.

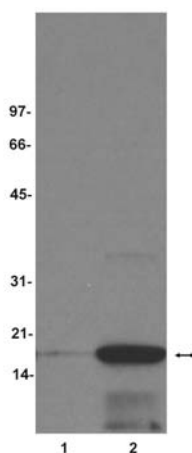
**Quantity:** 50 assays per kit.

**Storage and Stability:** Lyophilized: Stable for 2 years at -20°C from date of shipment, when stored with provided desiccant. Reconstituted: Stable for 6 months at -70°C.

**Rehydration:** Aseptically reconstitute to 1mg/ml with sterile water. Control Histones, untreated may be rehydrated in 2X RSB if used exclusively for immunoblotting. Aliquot to avoid repeated freezing and thawing.

#### Quality Control Testing

Immunoblot Analysis: Use 5-20µg per lane. 10µg of histones from untreated and colcemid-treated HeLa cells were used as a positive control for immunoblot analysis using 0.5µg/ml anti-phospho-Histone H3 (Ser10) (Catalog # 06-570) and 1:1000 dilution of anti-phospho-Histone H3 (Ser28) (Catalog # 07-145). 1µg/ml anti-phospho-Histone H1 (Catalog # 06-597) was used with a previous lot.



#### **Immunoblot Analysis**

Representative blot from a previous lot. 10µg Control Histones, untreated (lane1) or colcemid-treated (lane 2), were resolved by electrophoresis, transferred to nitrocellulose and probed with 0.5µg/ml anti-phospho-Histone H3 (Ser10) (Catalog # 06-570). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Histone H3 (17kDa).

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on **10 $\mu$ g Control Histones** and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
1. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween-20, (TBST-MLK) for 30-60 minutes at room temperature with constant agitation.
2. Incubate the nitrocellulose with an appropriate Histone-specific antibody, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
3. Wash the nitrocellulose twice with water.
4. Incubate the nitrocellulose with the appropriate secondary reagent in TBST-MLK for 1.5 hours at room temperature with agitation.
5. Wash the nitrocellulose with water twice.
6. Wash the nitrocellulose in TBS-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).