



cell signaling solutions

## Certificate of Analysis

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### Control Histones (+/- sodium butyrate)

(HeLa cell acid extract)

Catalog # 17-305

Lot # 25941

#### Kit Components

**Control Histones, untreated, (HeLa cell acid extract),** Catalog # 13-112, Lot # 25942. 10 vials, each vial containing **50µg** of precipitated core histones, lyophilized from sterile, distilled water. Lyophilized powder.

**Control Histones, sodium butyrate-treated, (HeLa cell acid extract),** Catalog # 13-113, Lot # 25943. 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 of untreated and sodium butyrate-treated HeLa cells.

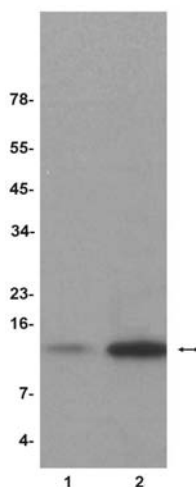
**Quantity:** 50 assays per kit.

**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, distilled water. Aliquot to avoid repeated freezing and thawing.

#### Quality Control Testing

Immunoblot Analysis: Use 5-20µg per lane. 10µg of histones from untreated and sodium butyrate-treated HeLa cells were used as a positive control for immunoblot analysis using 1µg/ml anti-acetyl Histone H4 (Catalog # 06-598), 0.2µg/ml anti-acetyl-Histone H3 (Catalog # 06-599) and 1:4000 dilution of anti-hyperacetylated Histone H4 (Penta) (Catalog # 06-946).



#### **Immunoblot Analysis**

Representative blot from a previous lot. 10µg Control Histones, untreated (lane 1) or sodium butyrate-treated (lane 2), were resolved by electrophoresis, transferred to nitrocellulose and probed with 2µg/ml anti-acetyl-Histone H4 (Catalog # 06-598). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates acetyl Histone H4 (10kDa).

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on **5-20 $\mu$ g Control Histones** and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20-30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with an appropriate Histone-specific antibody, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose with the appropriate secondary reagent in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).