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EZExtract™ Core Histone Isolation Kit

12/15

(Catalog # K234-100; 100 assays; Store at -20°C)

I. Introduction:

Histones are small alkaline proteins that interact with eukaryotic DNA to form the fundamental subunit of chromatin known as the nucleosome. The core histones H2A, H2B, H3 and H4 form the central spooling unit of the nucleosome; with two copies of each comprising a histone octamer that interacts with the negatively charged DNA. They enable an ordered compaction of the DNA, as well as spatiotemporal control of gene expression and genomic replication throughout mitosis. Post-translational modification of this core complex results in structural changes within the chromatin and epigenetic control of gene expression. BioVision's EZExtract™ Core Histone Isolation Kit offers an optimized suite of reagents to rapidly extract the total core histones while preserving acetylation from tissues and cultured cells for biochemical and epigenetic analyses. One hundred standard extractions at a scale of 10^7 cells or 100 mg tissues can be performed with the reagents in this kit. Yield of the total histone proteins is approximately 0.4 mg per 10^7 cells or 100 mg of tissue.

II. Applications:

- Isolation of intact functional core histones from tissues and cultured cells
- Protein profiling, post-translational modification and epigenetic analyses
- Western blot and ELISA

III. Sample Type:

- Mammalian tissues.
- Cultured cells

IV. Kit Contents:

Components	K234-100	Cap Code	Part Number
Lysis Buffer	2 X 100 ml	NM	K234-100-1
Extraction Reagent	25 ml	WM	K234-100-2
Neutralizing Buffer	10 ml	NM	K234-100-3
DTT (1 M)	100 µl	Green	K234-100-4

V. User Supplied Reagents and Equipment:

- Dounce Tissue Homogenizer (Cat # 1998)
- PBS Tablets (Cat # 2129)
- PMSF (Cat # 1548)

VI. Storage Conditions and Reagent Preparation:

Store the DTT at -20°C. Remaining reagents may be stored at 4° or -20°C. Please read the entire protocol before performing the assay.

- **Neutralization buffer**: If a precipitate is visible in the Neutralization Buffer, warm to 37°C in a water bath to dissolve it and chill on ice prior to use. Aliquot enough buffer for the number of assays to be performed, and add the appropriate amount of 1 M DTT for a final concentration of 1 mM DTT to the neutralization buffer right before use.
- **Extraction Reagent, Neutralizing Buffer and DTT (1M)**: Ready to use. Bring to room temperature (RT) before use.

VII. Total Core Histone Isolation Protocol:

1. Sample Preparation:

Wash: Harvest 1 x 10^7cells and wash twice with ice cold Phosphate Buffered Saline (PBS). Resuspend the pellet in 1 ml of PBS and transfer cells to a 1.5 ml tube. Spin cells at 600 X g for 10 minutes in a microfuge and aspirate supernatant. Cut 100 mg tissue of interest into 2 mm^3 sections and wash twice in a 1.5 ml tube with 1 ml of ice-cold PBS. Centrifuge the cells at 600 x g for 10 min for each wash step and discard the supernatant.

Lysis: Resuspend washed cells in 1 ml of ice-cold Lysis Buffer (optional: containing 2 mM PMSF) and lyse for 10 minutes on ice with intermittent gentle mixing (7-10 tube inversions). Centrifuge the lysate at 600 x g for 10 minutes at 4°C. Remove the supernatant and wash the pellet with 0.5 ml of Lysis Buffer. Stain 5 µl of cell lysate with Trypan Blue and view under a microscope at 20x on a glass slide. At least 80-90% of the cells should be lysed. Centrifuge the lysate and discard the supernatant. Repeat the wash step with 0.5 ml of Lysis Buffer and remove supernatant. Resuspend the washed tissue in 1 ml of ice-cold Lysis Buffer (optional: containing 2 mM PMSF) and homogenize it with a Dounce homogenizer on ice to fully disperse the cells. To check for the homogenization efficiency in the tissue sample, view the homogenized sample under a microscope. You should see a uniform suspension. Typically for soft tissues 10 - 15 strokes and for hard tissues 15 - 20 strokes are sufficient. Transfer the lysate into a 1.5 ml tube and incubate on ice for 10 minutes. Spin the minced tissue in a table top microfuge at 600 x g for 10 min. Remove the supernatant and wash with 0.5 ml of Lysis Buffer. Centrifuge as before and discard the supernatant.

Extraction: Completely resuspend pellet in 0.25 ml of ice-cold Extraction Reagent and incubate on ice for 1 hour. Centrifuge at 10000 x g for 10 minutes at 4°C and collect the supernatant. Add 0.1 ml ice-cold Neutralizing Buffer containing 1 mM DTT directly to the supernatant and mix well. This isolate contains the core histones. Quantify the histones isolated with any protein quantitation assay (Cat #s: K810-K814, K818, and K819). BSA can be used as a standard

Note: It is possible to scale down or up the sample amount by scaling up or down the volumes used in the protocol with varying yield results.

2. Storage Conditions based on Application:

Store histones at -20°C for up to one week (-80°C for longer storage). Avoid multiple freeze thaw cycles.

Note: If salt precipitates are seen in the extracts after being frozen, thaw extracts on ice and pipette gently several times until salts are re-dissolved.

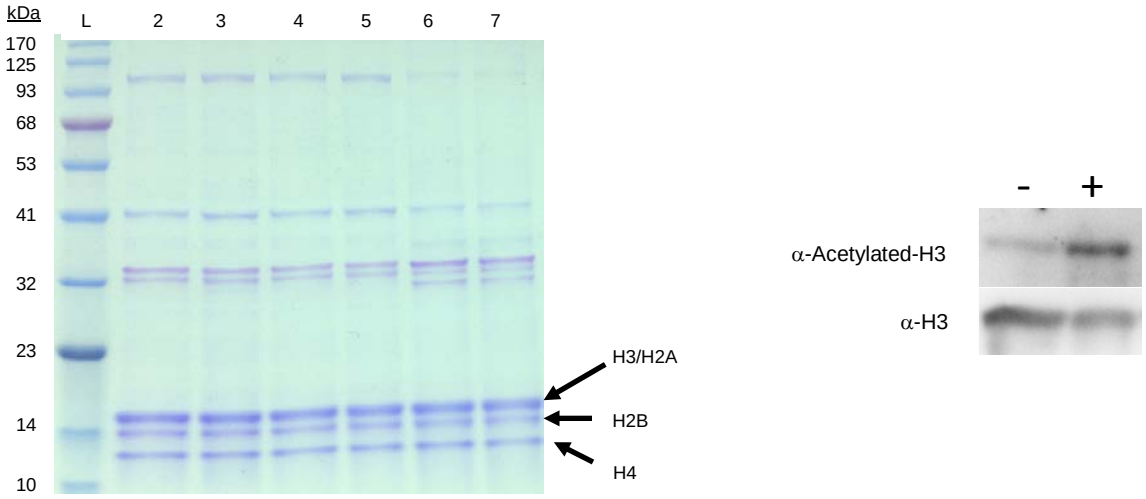

Figure: (a) Total core histones from HeLa cells and murine liver tissue were purified according to the above-mentioned protocol. 10 µg were loaded onto the 12% Bis-Tris gel. Lane 1: Protein Marker. Lanes 2 – 5: HeLa Core Histones; Lanes 6 -7: Murine Liver Core Histones (b) Western Blot of Acetylated Histone H3 extracted from synchronous HeLa cultured pre-washed with either PBS (-) or PBS containing BioVision EzAcetyl Preserve™ (Cat. 9558) (+). The top panel was probel with anti-acetylated-Histone H3 antibody (αAcetylated-H3 and the bottom panel was probed with anti –H3 (α-H3) as a loading control.

VIII. RELATED PRODUCTS:

Histone H2A Antibody (3621)
Acetyl-Histone H2A Antibody (3653)
Acetyl-Histone H2B Antibody (3654)
Histone H2AX Antibody (3761)
Histone H2B Antibody (3600-100)
Histone H3 (phospho-Ser28) (Clone 117C826) (6119) Antibody (6119)
Histone H4 Antibody (3624-100)
Histone H3 Antibody (3623)
Acetyl-Histone H4 Antibody (3656)
PBS (2129)
PMSF (1548-5)
Dounce Tissue Grinder (1998)

FOR RESEARCH USE ONLY! Not to be used on humans.