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Product Information

Core Histone Isolation Kit

Catalog Number **EPI024**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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. This 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 of tissue can be performed with the reagents in this kit. Yield of the total histone proteins is ~0.4 mg per 10^7 cells or 100 mg of tissue.

Components

The kit is sufficient for 100 assays.

Lysis Buffer Catalog Number EPI024A	2 × 100 mL
Extraction Reagent Catalog Number EPI024B	25 mL
Neutralizing Buffer Catalog Number EPI024C	10 mL
DTT (1 M) Catalog Number EPI024D	100 µL

Reagents and Equipment Required but Not Provided.

- Dounce tissue homogenizer
- PBS Tablets
- PMSF
- Trypan Blue

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Store the DTT solution at –20 °C. Remaining reagents may be stored at 4 °C or –20 °C.

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 (1 M) – Ready to use. Bring to room temperature (RT) before use.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Briefly centrifuge all small vials prior to opening.

Procedure
Please read the entire protocol before performing the sample preparation.

Cell Wash
Harvest 1×10^7 cells 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 × *g* for 10 minutes in a microfuge and aspirate supernatant.

Tissue Wash
Cut 100 mg of 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 × *g* for 10 minutes for each wash step and discard the supernatant.

Cell 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 × *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 20× 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.

Tissue Lysis
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. A uniform suspension should be observed. 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 × *g* for 10 minutes. 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 10,000 × *g* for 10 minutes at 4 °C and collect the supernatant. Add 0.1 mL of 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. 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.

Storage Conditions Based on Application
Store histones at –20 °C for up to one week (–70 °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.

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