Core Histone Isolation Kit

Cat. No. CHS-003K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	
Applications	Isolation of intact functional core histones from tissues and cultured cells Protein profiling, post-translational modification and epigenetic analyses Protein profiling of Histones by SDS-PAGE and Western blot
Storage	Store kit at -20centigrade. Briefly spin small vials prior to opening. Store histones at -20centigrade for up to one week (-80centigrade 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.
Kit Components	• Lysis Buffer 2 X 100 ml • Extraction Reagent 25 ml • Neutralizing Buffer 10 ml • DTT (1 M) 100 µl
Materials Required but Not Supplied	Dounce Tissue Homogenizer PBS Tablets PMSF
Preparation	Store the DTT at -20centigrade. Remaining reagents may be stored at 4centigrade or -20centigrade. Please read the entire protocol before performing the assay. • Neutralization buffer: If a precipitate is visible in the Neutralization Buffer, warm to 37centigrade in a water bath to dissolve it and chill on ice prior to use. Aliquot enough

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	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.
Separation Protocol	Total Core Histone Isolation Protocol: 1. Sample Preparation: Wash: Harvest 1 x 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 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 4centigrade. 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

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	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 4centigrade 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. 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.
Sample Type	• Mammalian tissues. • Cultured cells

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