Product Manual

.

EpiXtract™ Total Histone Extraction Kit (High Throughput)

Catalog #: ENZ-45013

2 x 96 Extractions

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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INTRODUCTION

Histones are the main proteins that help package DNA by acting as spools around which DNA can wind into structural units called nucleosomes. This act of winding or unwinding plays a significant role in gene regulation.

Core histones include H2A, H2B, H3, and H4. Histones undergo post-translational modifications, which alter their interaction with DNA and nuclear proteins. The H3 and H4 histones have long tails protruding from the nucleosome, which can be covalently modified at several sites. Modifications of the tail include methylation, acetylation, phosphorylation, ubiquitination, sumoylation, citrullination, and ADP-ribosylation (H2A can also be modified). Combinations of modifications are thought to constitute a code, which is referred to as the "histone code." Histone modifications play a key role in diverse biological processes such as gene regulation, DNA repair and chromosome condensation (mitosis).

The EpiXtract™ Total Histone extraction kit provides a simple and high throughput method for extracting histone proteins. These extracted histones can be used for a variety of histone modification applications (acetylation, methylation, and sumoylation).

The EpiXtract™ Total Histone extraction kit can be used to directly extract histones from mammalian cells cultured in a 96-well plate. It has the fastest procedure available. The assay can be completed within an hour using as little as 10^4 cells.

SAFETY WARNINGS AND PRECAUTIONS

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Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

REAGENTS PROVIDED AND STORAGE

The EpiXtract™ Total Histone Extraction Kit (HT) is shipped at ambient room temperature.

Upon receipt: (1) Store **DTT Solution** at 4°C; and (2) Store all remaining components at room temperature.

Reagent	Volume	Storage Temperature
Pre-Lysis Buffer	14mL	RT
Lysis Buffer	10mL	RT
Balance Buffer	6mL	RT
DTT Solution	20µL	4°C

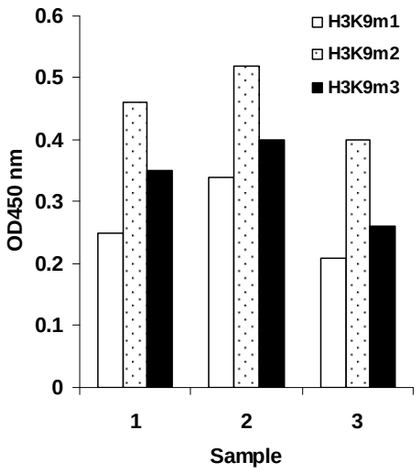
ADDITIONAL MATERIALS REQUIRED

- Adjustable pipette, multi-channel recommended
- Aerosol resistant pipette tips
- Plate centrifuge or a centrifuge with a plate carrier
- Tissue culture microplate
- PCR plate
- PCR plate seal film or strip caps
- Vortex
- Freshly made PBS solution

PRINCIPLE

The EpiXtract™ Total Histone Extraction Kit (High Throughput) simply applies proprietary histone isolation buffers to cells. After treatment with Pre-lysis, Lysis, and Balance buffers, the total histones are easily extracted for immediate use or storage at proper conditions.

Schematic procedure for using the EpiXtract™ Total Histone Extraction Kit (HT).



Histone extracts were prepared from MCF-7 cells (30,000cells/well) using the EpiXtract™ Total Histone Extraction Kit (HT) and acetyl histone H3 was quantified .

PROCEDURE

For the best results, please read the procedure in its entirety prior to starting your experiment.

Cell Lysis

1. Grow cells in the plate to desired density (40,000-50,000 cells/well).
2. Remove as much cell culture medium as possible.
3. Wash the cells 2 times by adding PBS wash buffer to each well, 200µl each time.
4. Remove as much residue wash buffer as possible.
5. Add 60µl of **Pre-Lysis Buffer** per well and incubate for 20 min at RT with occasional shaking every 5 min.

Histone Extraction

1. Collect cell lysate/solution from each well and transfer to a PCR plate correspondingly.
2. Cover the PCR plate with seal film or PCR strip caps.
3. Centrifuge at 1500rpm for 10 min at 4°C in a plate centrifuge or a centrifuge with a plate carrier.
4. Remove supernatant
5. Add 30µl of **Lysis Buffer** per well to re-suspend the pellet and incubate on ice for 30 min with occasional vortexing every 10 min.
6. Centrifuge at 3000 rpm for 10 min at 4°C and transfer the supernatant fraction (containing acid-soluble proteins) into a new PCR plate.
7. Prepare **Balance-DTT Buffer** by adding **DTT Solution** to **Balance Buffer** at a 1:500 ratio (e.g., 1µl of **DTT Solution** + 500µl of **Balance Buffer**).
8. Add 10µl of **Balance-DTT Buffer** to the 30µl of supernatant and mix by slightly shaking the plate.

Protein Concentration Measurement

Quantify the protein concentration with an OD reading. BSA can be used as a standard.

Histone Extraction Storage

Use immediately or cap the PCR plate with PCR strip caps and store the extract at –20°C for several days, or –80°C for long-term storage. Avoid repeated thawing and freezing.

Note*: If salt precipitates are seen in the extracts after being frozen, warm the extracts at room temperature for several minutes and pipette around several times until salts are re-dissolved.*

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