

EpiQuik™ Total Histone Extraction HT Kit

Base Catalog # OP-0007

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Total Histone Extraction HT Kit is suitable for a quick preparation of total histone extracts from mammalian cells in a 96 well plate format.

Input Amount: The minimal amount of starting materials can be as low as 10^4 cells. For the best results, the cell number should be greater than 3×10^4 cells. A total of 2 X 96 standard extractions (use 3×10^4 cells) can be performed with this kit.

Yield: Yield of the total histone proteins can be up to 400 ng per 10^4 cells. The yield may vary depending on the cell type.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the plate wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

KIT CONTENTS

Component	2 X 96 extractions Cat. # OP-0007-192	Storage Upon Receipt
Pre-Lysis Buffer	14 ml	RT
Lysis Buffer	10 ml	RT
Balance Buffer	6 ml	RT
DTT Solution	20 µl	4°C

SHIPPING & STORAGE

The EpiQuik™ Total Histone Extraction HT Kit is shipped at ambient room temperature.

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

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- 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
- PBS solution

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ Total Histone Extraction HT Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiQuik™ Total Histone Extraction HT Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ Total Histone Extraction HT Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

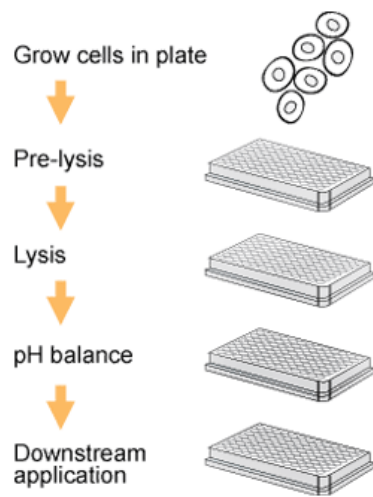
Histones are the chief protein components of chromatin in biology. They act as spools around which DNA winds, and also play a role in gene regulation.

The core histones include H2A, H2B, H3, and H4. Histones undergo posttranslational 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 places. 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, the so-called "histone code." Histone modifications act in diverse biological processes such as gene regulation, DNA repair and chromosome condensation (mitosis).

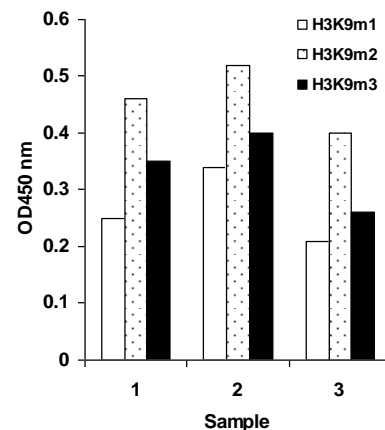
The EpiQuik™ Total Histone Extraction HT Kit provides a simple and high throughput method for extracting histone proteins used for a variety of applications, which include histone modifications such as acetylation, methylation, and sumoylation. This kit is also specifically designed to meet the requirements of histone extracts used in EpiQuik™ histone quantification assays. The kit can be used to directly extract histones from mammalian cells cultured in a 96-well plate. The EpiQuik™ Total Histone Extraction HT Kit has the fastest procedure available on the market, allowing completion within 60 minutes.

PRINCIPLE & PROCEDURE

The EpiQuik™ Total Histone Extraction HT Kit simply applies our 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 of the EpiQuik™ Total Histone Extraction HT Kit.



Histone extracts were prepared from MCF-7 cells (30,000cells/well) using the EpiQuik Total Histone Extraction HT Kit and acetyl histone H3 was quantified using the EpiQuik Global Pan-Methyl Histone H3K9 Quantification Kit (Colorimetric) (Cat. #P-3036).

PROTOCOL

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

1. Cell Lysis

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

2. Histone Extraction

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

3. Protein Concentration Measurement

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

4. 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.*

RELATED PRODUCTS

Protein Preparation

OP-0002

OP-0022

OP-0003

OP-0006

EpiQuik™ Nuclear Extraction Kit

EpiQuik™ Nuclear Extraction Kit II (Nucleic Acid-Free)

EpiQuik™ Whole Cell Extraction Kit

EpiQuik™ Total Histone Extraction Kit