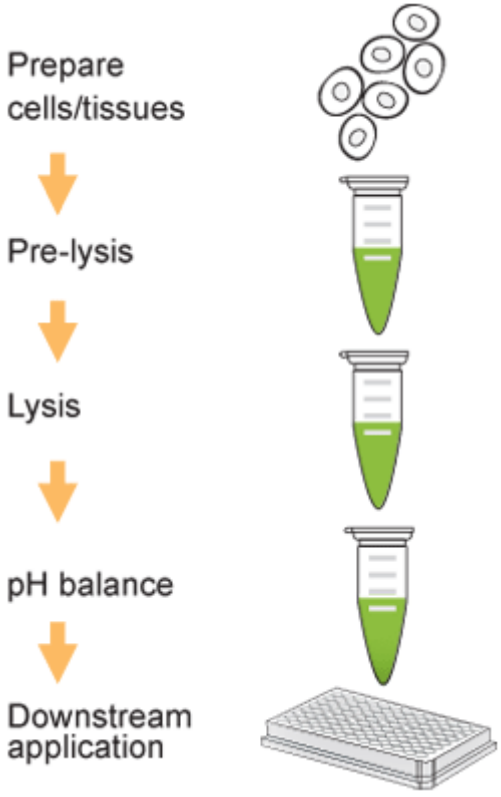
# EpiQuik™ Total Histone Extraction Kit for Tissues (Treated and Untreated Version 3

**Natalie Crowley**


## Abstract

*The EpiQuik™ Total Histone Extraction Kit* is a complete set of optimized buffers and reagents for extracting total core histone proteins (H2A, H2B, H3, and H4) from mammalian cells or tissues in a simple 60 minute procedure. The post-translational modifications (PTM) in the histone extracts are kept intact and thus can be used with Epigentek's histone modification assay kits or in a variety of downstream applications for histone methylation, acetylation, phosphorylation, sumoylation, ubiquitination, citrullination, and ADP-ribosylation studies.

- Pre-optimized and simple 1 hour protocol.
- Conveniently includes all essential reagents to carry out a histone extraction.
- Standardized procedure for reproducible results.
- Extracts a high yield of total core histones from as little as 1 mg of tissues.
- Post-translational modifications are kept intact.
- Does not affect histone modification status or levels.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## Guidelines

### PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

**Uses**: The EpiQuik™ Total Histone Extraction Kit is suitable for a quick preparation of total histone extracts from mammalian cells and tissue samples.

**Input Amount**: The minimal amount of starting materials can be as low as $10^5$ cells or 1 mg of tissue. For the best results, the cell number should be greater than $10^6$ cells or the tissue amount should be greater than 10 mg. A total of 100 standard extractions (use $10^7$ cells or 100 mg of tissue per extraction) can be performed with this kit.

**Yield**: Yield of the total histone proteins can be up to 0.4 mg per $10^7$ cells or per 100 mg of tissue. The yield may vary depending on the cell or tissue type.

**Precautions**: To avoid cross-contamination, carefully pipette the sample or solution into the strip 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 | 100 extractions<br>Cat. #<br>OP-0006-100 | Storage<br>Upon<br>Receipt |
|---|---|---|
| 10X Pre-Lysis Buffer | 10 ml | RT |
| Lysis Buffer | 20 ml | RT |
| Balance Buffer | 8 ml | RT |
| DTT Solution | 20 µl | 4°C |
| User Guide | 1 | |

### SHIPPING & STORAGE

The EpiQuik™ Total Histone Extraction 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.

## GENERAL PRODUCT INFORMATION

**Quality Control**: Each lot of the EpiQuik™ Total Histone Extraction 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. Thus, only use the User Guide that was supplied with the kit when using that kit.

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

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

## PRINCIPLE & PROCEDURE

The EpiQuik™ Total Histone Extraction Kit simply applies our proprietary histone isolation buffers to cells or tissues. After treatment with pre-lysis, lysis, and balance buffers, the total histones are easily extracted for immediate use or storage at proper conditions.

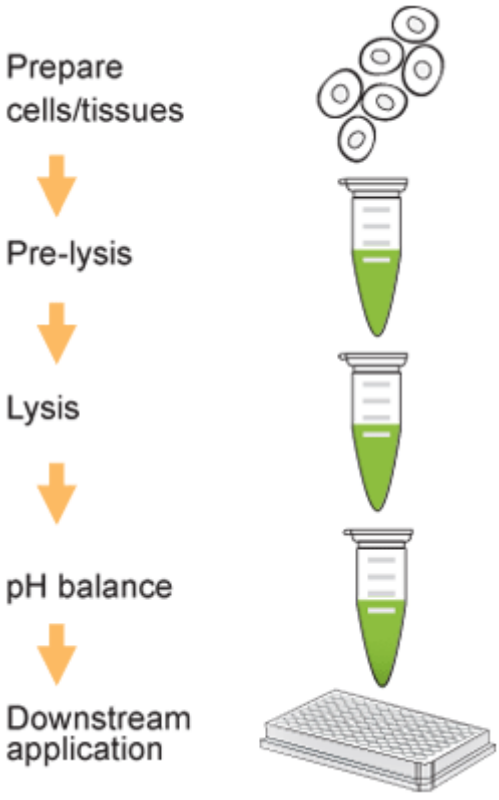


**Fig. 1**. Schematic procedure of chromation isolation with the EpiQuik™ Total Histone Extraction Kit.

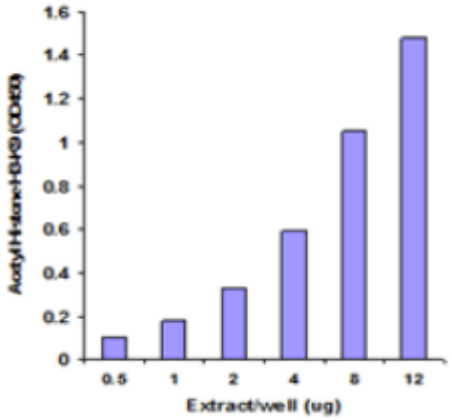


**Fig. 2.** Histone extracts were prepared from MCF-7 cells using the EpiQuik Total Histone Extraction Kit and acetyl histone H3-K9 was quantified using the EpiQuik Global Acetylated Histone H3-K9 Quantification Kit (Colorimetric) (Cat. #P-4010).

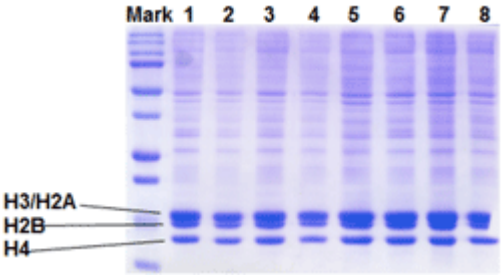


**Fig. 3.** SDS-PAGE analysis of histone extracts was prepared with the EpiQuik™ Total Histone Extraction Kit. 10 µg of each sample were loaded per lane (1-8).

## Materials

EpiQuik Total Histone Extraction Kit OP-0006-100 by EpiGentek

## Protocol

### Step 1.

Weigh the sample and cut the sample into small pieces (1-2 mm3 ) with a scalpel or scissors.

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

### Step 2.

Transfer tissue pieces to a Dounce homogenizer.

**Step 3.**

Dilute **10X Pre-Lysis Buffer** into **1X Pre-Lysis Buffer** with distilled water at a 1:10 ratio (e.g., 1 ml of **10X Pre-Lysis Buffer** + 9 ml of water).

**Step 4.**

Add the Diluted **1X Pre-Lysis Buffer** at 1 ml per 200 mg of tissue, and disaggregate tissue pieces by 50-60 strokes.

**Step 5.**

Transfer homogenized mixture to a 15 ml conical tube and centrifuge at 3000 rpm for 5 min at 4°C.

DURATION
00:05:00

**Step 6.**

**If** total mixture volume is less than 2 ml, transfer mixture to a 2 ml vial and centrifuge at 10,000 rpm for 1 min at 4°C.

DURATION
00:01:00

**Step 7.**

Remove supernatant.

**Step 8.**

Re-suspend cell/tissue pellet in 3 volumes (approximately 200 µl/$10^7$ cells or 100 mg of tissue) of **Lysis Buffer** and incubate on ice for 30 min.

DURATION
00:30:00

**Step 9.**

Centrifuge at 12,000 rpm for 5 min at 4°C and transfer the supernatant fraction (containing acid-soluble proteins) into a new vial.

DURATION
00:05:00

**Step 10.**

- Prepare **Balance-DTT Buffer** by adding **DTT Solution** to **Balance Buffer** at a 1:500 ratio (e.g., 1 µl of **DTT Solution** + 500 µl of **Balance Buffer**).
- Add 0.3 volumes of the **Balance-DTT Buffer** to the supernatant immediately (e.g., 0.3 ml of **Balance-DTT Buffer** to 1 ml of supernatant).

**Step 11.**

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

**Step 12.**

Aliquot and store the extract at –20°C for several days, or –80°C for long-term storage. Avoid repeated thawing and freezing.

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***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.*

## Warnings

To avoid cross-contamination, carefully pipette the sample or solution into the strip 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