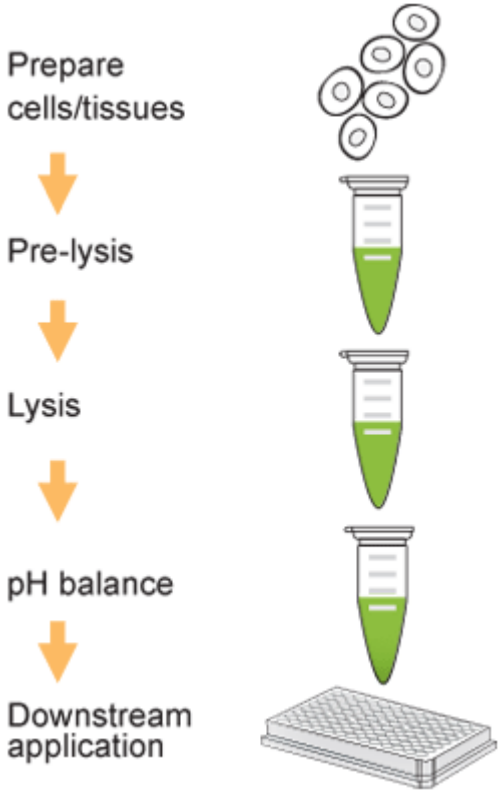
EpiQuik™ Total Histone Extraction Kit Version 2

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.



Citation: Natalie Crowley EpiQuik™ Total Histone Extraction Kit. **protocols.io** dx.doi.org/10.17504/protocols.io.fvzbn76
Published: 22 Sep 2016

Protocol

Step 1.

For Tissues (Treated and Untreated)

- Weigh the sample and cut the sample into small pieces (1-2 mm3) with a scalpel or scissors.
- Transfer tissue pieces to a Dounce homogenizer.
- 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).
- Add the Diluted 1X Pre-Lysis Buffer at 1 ml per 200 mg of tissue, and disaggregate tissue pieces by 50-60 strokes.
- Transfer homogenized mixture to a 15 ml conical tube and centrifuge at 3000 rpm for 5 min at 4°C. 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.
- Remove supernatant.

For Cells (Treated and Untreated)

- Harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 min at 4°C.
- 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).
- Re-suspend cells in the Diluted 1X Pre-Lysis Buffer at 107 cells/ml and lyse cells on ice for 10 min with gentle stirring.
- Centrifuge at 3000 rpm for 5 min at 4°C. If cell lysates are prepared in a 1.5 to 2 ml size vial, centrifuge at 10,000 rpm for 1 min at 4°C.
- Remove supernatant.

Step 2.

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.

Step 3.

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

Step 4.

- 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 5.

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

Step 6.

Aliquot 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.