ab113476 – Histone Extraction Kit

For the extraction of histone proteins from mammalian cells and tissue

For overview, typical data and additional information please visit: www.abcam.com/ab65333
(use www.abcam.cn/ab65333 for China, or www.abcam.co.jp/ab65333 for Japan)

This product is for research use only and is not intended for diagnostic use

Storage and Stability: **Store kit as given in the table upon receipt.**

All components of the kit are stable for 6 months from the date of shipment or expiry date.

Check the Buffers to see if they contain salt precipitates before use. If so, warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.

Materials Supplied:

Item	Quantity	Storage temperature
10X Pre-Lysis Buffer	10 mL	Ambient
Lysis Buffer	20 mL	Ambient
Balance Buffer	8 mL	Ambient
DTT Solution	20 µL	4°C

1) 1X Pre-Lysis Buffer could be stable for 6 months at RT.
2) Balance-DTT Buffer (DTT Solution + Balance Buffer) could be stable for at least 6 months if stored at -20°C.

Materials Required, Not Supplied

These materials are not included, but will be required to successfully perform this assay:

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Pipette tips
- 1.5 mL microcentrifuge tubes
- Vortex mixer
- Dounce homogenizer with small clearance pestle
- Scapel or Scissors
- Thermocycler with 48 or 96-well block
- Centrifuge (up to 14,000 rpm)
- Orbital shaker
- 15 mL conical tube
- Cells or tissues
- Distilled Water
- Protein determination method

Limitation:

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

Technical Hints:

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

Prepare fresh reagents immediately prior to use.

1. **1X Pre-Lysis Buffer:**
   Dilute 1 mL of 10X Pre-Lysis Buffer with 9 mL of distilled water.

2. **Balance-DTT Buffer:**
   Add 1 µL of DTT Solution to 500 µL of Balance Buffer.

Sample Preparation

Tissues (treated or untreated)

1. Weigh the sample and cut it into small pieces (1-2 mm^3) with a scalpel or scissors.
2. Transfer the tissue pieces to a Dounce homogenizer.
3. Add 1X Pre-Lysis buffer so that the tissue is at 200 mg/mL and disaggregate tissue pieces by 50-60 strokes
4. 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.
5. Remove supernatant.

Cells (treated or untreated)

1. Harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 minutes at 4°C.
2. Re-suspend cells in the 1X Pre-Lysis Buffer at 10^7 cells/mL and lyse cells on ice for 10 min with gentle stirring.
3. 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.
4. Remove supernatant.

Note: After treatment with **Pre-Lysis Buffer**, the supernatant will contain the cytoplasmic fraction, while the intact nuclei will pellet.

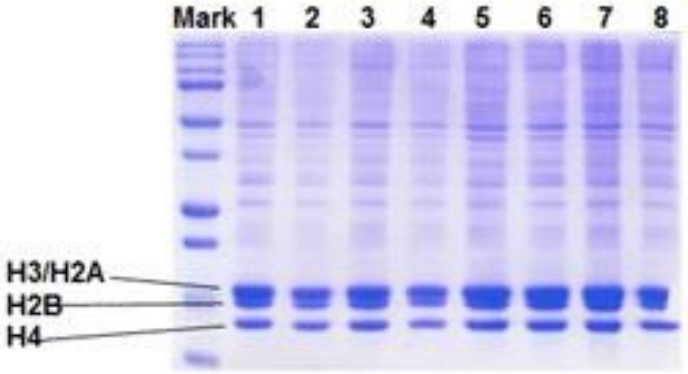
Extraction Protocol:

5. 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.

6. Centrifuge at 12,000 rpm for 5 min at 4°C and transfer the supernatant fraction (containing acid-soluble proteins) into a new vial.
7. 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).
8. Quantify the protein concentration with an OD reading. BSA can be used as a standard.
9. Aliquot and store the extract at -20°C for several days, or -80°C for long-term storage. Avoid repeated thawing and freezing.

ΔNote: 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.

Analysis



SDS-PAGE analysis of histone extracts was prepared with ab113476 Histone Extraction Kit. 10 µg of each sample were loaded per lane (1-8).

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)