ab113476 – Histone Extraction Kit

Instructions for Use

For the extraction of histone proteins from mammalian cells and tissue

View kit datasheet: www.abcam.com/ab113476
(use www.abcam.cn/ab113476 for China, or www.abcam.co.jp/ab113476 for Japan)

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

Version 5 Last Updated 21 January 2019

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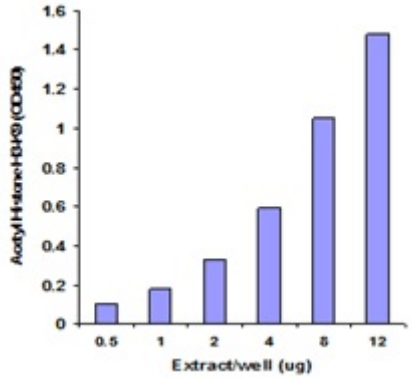
1. BACKGROUND

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).

ab113476 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. ab113476 is also specifically designed to meet the requirements of histone extracts used in histone quantification assays. ab113476 can be used to extract histones from mammalian cells and tissues. ab113476 allows for completion within 60 minutes.

ab113476 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.

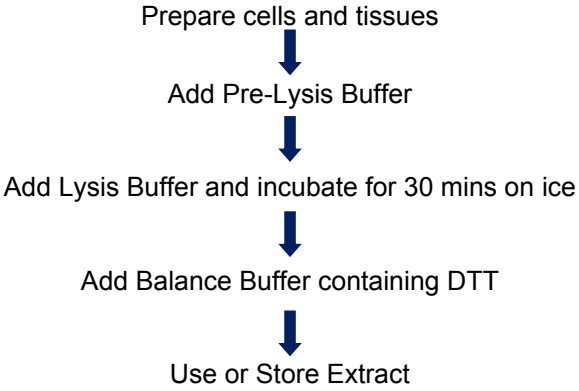


Histone extracts were prepared from MCF-7 cells using ab113476 and acetyl histone H3-K9 was quantified.

ab113476 is suitable for a quick preparation of total histone extracts from mammalian cells and tissue samples. 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 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.

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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. 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.

Observe the storage conditions for individual prepared components in sections 9 & 10.

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.

5. MATERIALS SUPPLIED

Item	100 Tests	Storage Condition (Before Preparation)
10X Pre-Lysis Buffer	10 mL	4°C
Lysis Buffer	20 mL	4°C
Balance Buffer	8 mL	4°C
DTT Solution	20 µL	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize 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

7. LIMITATIONS

- 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

8. 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.**

9. REAGENT PREPARATION

Prepare fresh reagents immediately prior to use.

9.1 **1X Pre-Lysis Buffer**

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

9.2 **Balance-DTT Buffer**

Add 1 µL of DTT Solution to 500 µL of Balance Buffer.

10. SAMPLE PREPARATION

Tissues (treated or untreated)

10.1.1 Weigh the sample and cut it into small pieces (1-2 mm^3) with a scalpel or scissors.

10.1.2 Transfer the tissue pieces to a Dounce homogenizer.

10.1.3 Add 1X Pre-Lysis buffer so that the tissue is at 200 mg/mL and disaggregate tissue pieces by 50-60 strokes

10.1.4 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.

10.1.5 Remove supernatant.

10.2 **Cells (treated or untreated)**

10.2.1 Harvest cells and pellet the cells by centrifugation at 1000 rpm for 5 minutes at 4°C.

10.2.2 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.

10.2.3 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.

10.2.4 Remove supernatant.

11. EXTRACTION PROTOCOL

11.1 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.

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

11.3 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).

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

11.5 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*.

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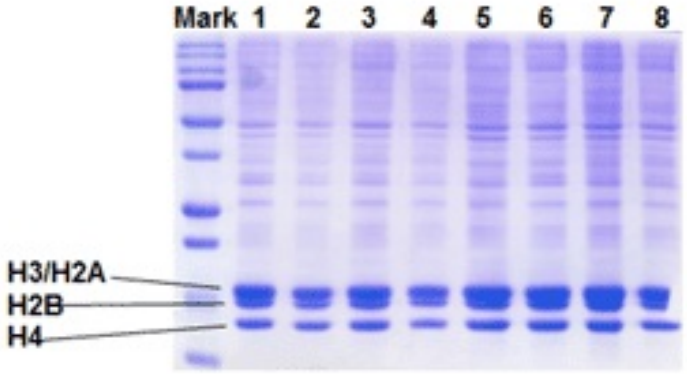
12. ANALYSIS



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

13. NOTES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp