Histone Purification Mini Kit

(version B4)

Catalog No. 40026

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The manufacturer of this documentation is Active Motif, Inc.

The purification method used in this kit is covered under US patent 8,163,481.

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Introduction

Active Motif's Histone Purification Mini Kit enables you to purify the core histone proteins and enrich for histone fractions while preserving their post-translational modifications (*e.g.* acetylation, methylation, and phosphorylation). This purification method is an improvement over acid precipitation methods and utilizes a convenient spin column and a proprietary buffer system to purify the core histones from cells and tissue samples. Histones isolated by this method are suitable substrates for downstream assays and *in vitro* chromatin assembly.

Unlike standard acid extraction techniques, this kit uses proprietary technology to purify the core histones. Post-translational modifications such as phosphorylation, acetylation and methylation are preserved, so you can extract core histone proteins from your cell culture or animal model and determine which modifications are present.

The Histone Purification Mini Kit provides reagents for 20 histone purifications from as little as $8x10^5$ cells up to grams of tissue due to the robust histone binding capacity of the purification column. The kit method is simple: first, an extract is made and applied to the purification column, then histones are eluted, enabling the purification of core histones. Histones may be quantified by an OD reading or quantitated empirically on a gel by comparison with histone standards.

product	format	catalog no.
Histone Purification Mini Kit	20 rxns	40026

The Histone Purification Mini Kit is for research use only. Not for use in diagnostic procedures.

Kit Performance

Core Histones Purified from Cells and Tissue

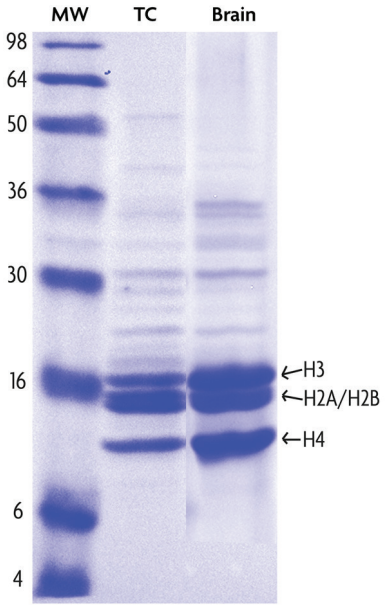
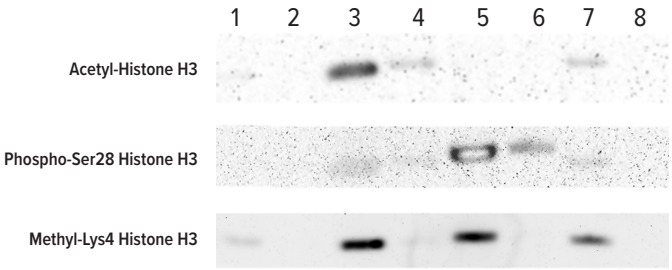


Figure 1: SDS-PAGE of histone fractions purified using the Histone Purification Mini Kit.

Ten µg per lane of core histones purified from logarithmically growing tissue culture cells (TC) and core histones isolated from rat brain tissue (Brain) were loaded and run on a 16% Tris-glycine gel.

Histone Purification Mini Kit preserves post-translational modifications

Active Motif's Histone Purification Mini Kit preserves phosphoryl, acetyl and methyl post-translational modifications on histones. The Western blot data shown below of the first and second elutions of unstimulated HeLa (lanes 1-2), sodium butyrate-treated HeLa (lanes 3-4), paclitaxel-treated HeLa (lanes 5-6) and rat brain histones (lanes 7-8) demonstrates that post-translational modifications are intact following purification of histones.



Yield of Histones:

The following yields are approximate. Results may vary according to cell or tissue type. It is possible to obtain a decent yield (10 µg) of histones from as few as 8 x 10^5 mammalian cells.

Adherent Cells: 0.1 mg total core histones from 8 x 10^6 cells (one 150 mm plate).

Suspension Cells: 0.1 mg total core histones from 8 x 10^6 cells.

Tissue: 1 mg histone per gram of tissue*

* The theoretical binding limit for these columns is 1 mg, but this has not been confirmed empirically. Loading more than the theoretical limit is not recommended.

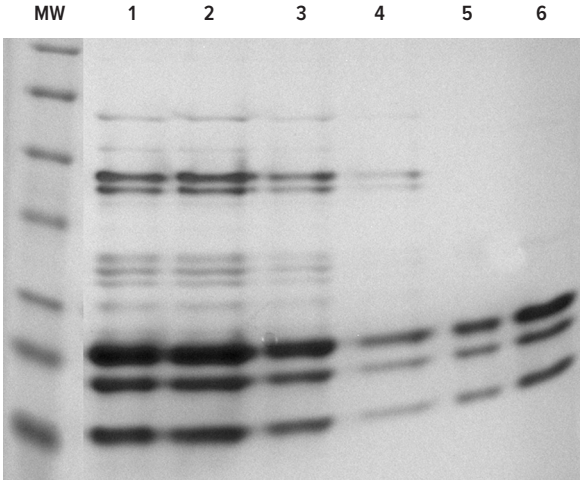


Figure 2: Analysis of histone yield using varying numbers of HeLa cells.

Histones were extracted from different numbers of HeLa cells using the Histone Purification Mini Kit and loaded onto a 16% SDS-PAGE gel. Purified HeLa core histones were loaded as a standard in parallel. On this particular gel system, H2A and H2B exhibit coincident migration.

Lane 1: 15 µl of a 100 µl elution from histones purified from a single 150 mm tissue culture plate of HeLa cells at 100% confluency (8 x 10^6 cells).

Lane 2: 15 µl of a 50 µl elution from histones purified from half of the cells from a 150 mm tissue culture plate of HeLa cells at 100% confluency (4 x 10^6 cells).

Lane 3: 15 µl of a 50 µl elution from histones purified from one-fifth of the cells from a 150 mm tissue culture plate of HeLa cells at 100% confluency (1.6 x 10^6 cells).

Lane 4: 15 µl of a 50 µl elution from histones purified from one-tenth of the cells from a 150 mm tissue culture plate of HeLa cells at 100% confluency (8 x 10^5 cells).

Lane 5: 2.5 µg of purified HeLa core histones.

Lane 6: 5 µg of purified HeLa core histones.

Kit Components and Storage

Please store each component at the temperature indicated below. All components are guaranteed stable for 6 months from date of purchase when stored at the appropriate temperatures.

Reagents	Quantity	Storage / Stability
5X Neutralization Buffer	50 ml	4°C
Extraction Buffer	100 ml	4°C
Equilibration Buffer	10 ml	4°C
Histone Wash Buffer	30 ml	4°C
Histone Elution Buffer	10 ml	4°C
Purification Spin Columns	20	Room Temp
Collection Tubes	40	Room Temp

Additional materials required

- Dounce homogenizer with a small clearance pestle (*e.g.* Active Motif cat. nos. 40401 or 40415; the large pestles supplied with these Dounces can be used for initial sample reduction, while the small pestles should be used to process the final homogenate).
- 1.7 ml microcentrifuge tubes
- Sterile water or Tris-EDTA, pH 8.0
- SDS sample buffer
- SDS-PAGE and Western blot reagents
- For precipitation of histones: Perchloric acid, 70% (Acros Organics, part no. 424030010)
- For washing of histones:
  4% perchloric acid
  0.2% HCl in acetone*
  Cold 100% acetone (VWR, part no. BDH1101)

* Add 0.5 ml of HCl stock (36.8%, Sigma, part no. H-7020) and adjust the volume with acetone until 92 ml. Store in a glass bottle at 4°C.

Protocol

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Step 1: Prepare Sample Extracts

For adherent cells:

1. Grow cells to 80-90% confluence in the appropriate medium. Discard media and wash the cells 2 times with pre-warmed (37°C) serum-free media: use 2 ml for a 35 mm dish; 10 ml for a 100 mm dish; or 20 ml for a 150 mm dish.
2. After the 2nd wash, aspirate any remaining wash media and add ice-cold Extraction Buffer to each dish: use 0.3 ml for each 35 mm dish; 0.8 ml for a 100 mm dish; or 1.5 ml for a 150 mm dish. Using a plastic scraper and a pipet, collect the cell protein extracts in a 1.7 ml microcentrifuge tube or a 15 ml tube as dictated by the amount of material. Pipet the cells up and down to homogenize them into the solution.

For suspension cells:

1. Grow cells to about 80-90% confluence in appropriate medium. If necessary, gently scrape the cells from the sides of the dish while keeping them in their media.
2. Pipet the cells with the media and transfer to a 15 ml tube.
3. Centrifuge at 1,000 x *g* for 5 minutes at room temperature.
4. Wash the cells twice with 20 ml pre-warmed (37°C) serum-free media. After each wash, centrifuge as in No. 3 above, then discard the serum-free media.
5. Resuspend the cells in ice-cold Extraction Buffer (0.5 ml per 150 cm^2 flask yields good concentrated extracts). Pipet up and down to homogenize the cells well, then transfer the resuspended cells to a microcentrifuge tube.

For tissues:

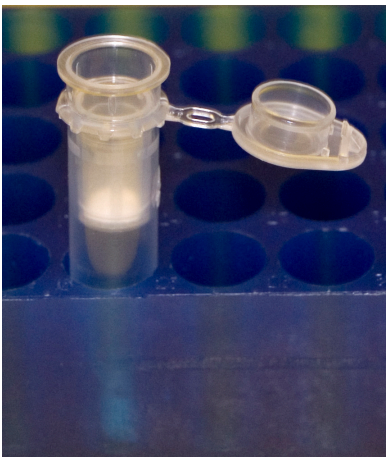
1. Homogenize the tissue completely in an ice-cold Dounce homogenizer using as little ice-cold Extraction Buffer as possible, as this will help ensure a highly concentrated extract. Keep the homogenate on ice.
2. Transfer the homogenate into a fresh microcentrifuge tube. Continue with Step 2: Prepare Crude Core Histone Extract on page 6.

Step 2: Prepare Crude Core Histone Extract

1. Leave the cells in Extraction Buffer for 30 minutes to 2 hours (or overnight) on a rotating platform at 4°C. (Time course experiments performed on some cell lines have shown that most histones are extracted in as little as 10 minutes).
2. Transfer the cell extracts to fresh tubes and centrifuge at maximum speed in a microfuge for 5 minutes at 4°C.
3. Transfer the supernatant, which contains the crude histones, to a new tube. The pellet can be discarded or stored at -20°C for future analysis. If the pellet is to be stored, neutralize it by adding 100 µl of 1 M Tris HCl pH 8.0.
4. Store the crude histones at -80°C, or continue with the next step.
5. Neutralize and equilibrate the crude histones with 1/4 volume of 5X Neutralization Buffer. For example, add 0.25 ml of 5X Neutralization Buffer to 1 ml crude histones, 0.5 ml (5X) to 2 ml, 1 ml (5X) to 4 ml of crude histones, *etc*.
6. Check the pH. If still acidic, add additional 5X Neutralization Buffer until the pH reaches 8.
7. It is recommended to remove an aliquot of the crude histones for comparison to the purified histones (column input control).

Step 3: Purify Core Histones

1. Equilibrate the spin columns by adding 0.5 ml Equilibration Buffer then centrifuging at 2,000 x *g* (approximately 4,500 rpm) for 5 minutes at 4°C.



2. Add 0.5 ml of the sample from Step 2 to the column and centrifuge at 2,000 x *g* for 5 minutes at 4°C. Remove the flow-through and save to a new 1.7 ml microcentrifuge tube. Repeat this step as many times as necessary to load the entire sample on the column. (The flow-throughs can be combined and analyzed to determine column binding efficiency.)
3. Wash the column with 0.5 ml Histone Wash Buffer and centrifuge at 2,000 x *g* for 5 minutes

at 4°C. Remove the wash buffer to a microfuge tube and save for analysis later. Repeat this step twice for a total of three washes.

4. Transfer the column to a new 2 ml round-bottom microcentrifuge tube. Add 100 µl of Histone Elution Buffer and centrifuge at 2,000 x *g* for 5 minutes at 4°C. Save the flow-through containing the eluted histone proteins.
5. **Optional second elution:** repeat step 4, if desired, keeping the 2nd eluate separate from the first eluate.

At this point the proteins are ready for use, but contain high levels of salts in the buffer that may interfere with some downstream binding assays or other applications. If desired, the proteins can be used without precipitation after being desalted using columns designed for this purpose (*e.g.* Zeba spin columns, Thermo Fisher part no. 89889). To further concentrate the proteins and remove the salt, proceed to Step 4: Precipitate Histone Proteins.

Step 4: Precipitate Histone Proteins

1. Precipitate the histone proteins overnight at 4°C by adding perchloric acid to a final concentration of 4%. For example add 6 µl of 70% perchloric acid to 100 µl fraction, then vortex.
2. On the following day, spin the samples at maximum speed in a microfuge for 1 hour at 4°C.
3. Gently wash the histone pellet with 1 ml cold 4% perchloric acid. Do not vortex the pellet. Centrifuge for 5 minutes at maximum speed in a microfuge. Repeat this step for a total of two washes.
4. Wash the histone pellet two times as in step 3 using ice cold acetone containing 0.2% HCl.
5. Wash the histone pellet two times as in step 3 using ice cold acetone.
6. Air dry the pellet until it is completely dry, around 10 to 20 minutes.
7. Resuspend in sterile water or TE. Flick the bottom of the tube gently with a finger. Let the pellet resuspend 10 to 20 minutes at room temperature, then gently vortex and centrifuge briefly. Check to make sure that pellet is completely resuspended. Store at -20°C or -80°C.

Step 5: Quantify Yield of Histone Proteins

1. Total core histone proteins can be quantified by measuring the absorbance at 230 nm. An OD of 0.42 from a sample diluted 1:10 indicates a protein concentration of 1 mg/ml.

   Undiluted samples may be outside the linear range of your spectrophotometer. Therefore, we recommend preparing a 1/10 dilution of your samples before quantifying.

$$\text{OD of } 0.42 = 1 \text{ mg / ml}$$

$$\frac{\text{OD } 0.42}{1 \text{ mg/ml}} = \frac{\text{Your OD}}{x \text{ mg / ml}}$$

Solve for x = Concentration of diluted stock
Multiply by 10 = Actual histone concentration

Appendix

Section A: Troubleshooting Guide

Problem/question	Recommendation
Clogging problems appear when loading the sample onto the column.	Perform an additional 5 minute centrifugation step at 2,000 x g.
In the precipitation step, there is no visible pellet after spinning the histone samples.	In general, a white pellet is visible. Leave some of washing solutions to avoid disturbing the pellet; do not vortex it. Also, the histones will not precipitate well if the preparation is too dilute. Elute the sample in less volume to obtain more concentrated preparations.
Is it possible to eliminate H1 from the final preparation of histones?	Yes. Precipitate the histone proteins in perchloric acid 4% (H1 protein remains soluble). H1 protein can be recovered by re-precipitation of the soluble fraction using 20-30% trichloroacetic acid.
Do the histone proteins react with Coomassie dye while in solution?	No. Core histones react poorly with Coomassie dye while in solution, and H1 does not react at all. However, H1 and core histones are stained by Coomassie effectively in gel.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

Active Motif North America

Toll free: 877.222.9543
Direct: 760.431.1263
Fax: 760.431.1351
E-mail: tech_service@activemotif.com

Active Motif Europe

UK Free Phone: 0800/169 31 47
France Free Phone: 0800/90 99 79
Germany Free Phone: 0800/181 99 10
Direct: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan

Direct: +81 (0)3 5225 3638
Fax: +81 (0)3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China

Direct: (86)-21-20926090
Cell Phone: 18521362870
E-mail: techchina@activemotif.com

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