

Acidic extraction of histone proteins

Histones are highly basic proteins that can be selectively extracted from cells, nuclei, or chromatin using dilute acids—a method first described by Kossel in 1884. This modernized protocol, adapted from Holt et al. (2021), enables efficient recovery of histones from small or precious samples.

The protocol uses acid extraction followed by trichloroacetic acid (TCA) precipitation to isolate total histones while minimizing loss of many post-translational modifications. It is particularly suited for downstream applications including Western blotting, immunoprecipitation, and LC-MS/MS proteomics.

Risk assessment

- Work with highly flammable liquids
- Work with concentrated acids
- ▷ Handle acids and flammable liquids in a certified chemical fume hood
- ▷ Wear gloves, safety glasses, lab coat
- Collect acetone as ORGANIC SOLVENT WASTE



Reviewed: Oct 26, 2023

Procedures

>> Acid extraction

- 0.2 M Hydrochloric acid, 1 mL (R)
- 100% Trichloroacetic acid
- 99.9% Acetone, 1 mL (R)
- 100% Acetone

- (1.) Start with a nuclei pellet or a nuclear fraction isolated from approximately 1.0×10^6 – 1.0×10^7 cells. Approximate the packed cell volume; this is 1 vol.

Hint: Continuous cell lines such as HeLa, U2OS, or 293T yield sufficient material from 1×10^6 cells, while 1.0×10^7 cells may be required for finite cell strains or primary cells.

- (2.) Gently vortex the pellet from the preceding nuclear isolation or extraction procedure.
- (3.) **Critical:** In a dropwise manner, add 5 vol (10 vol if starting with fewer than 1×10^6 cells) of 0.2 M hydrochloric acid to the pellet at 4 °C. Gently pipette up and down to disperse the pellet.

Critical: Add slowly to avoid rapid precipitation. If working with small volumes, pipette gently rather than dropwise.

- (4.) Incubate on ice for 5 min.

Note: Precipitation occurs rapidly; there is no benefit in increasing the duration of this step. However, extended incubation up to several hours will neither affect results, making this a reasonable break point.

- (5.) Centrifuge at $15\,000 \times g$ for 5 min at 4 °C. Transfer the supernatant to a clean tube.

This is why: The supernatant contains histones and basic proteins. The pellet contains non-histone nuclear proteins.

- (6.) To the supernatant, add 0.25 vol of 100% trichloroacetic acid. Vortex vigorously to precipitate histones.

Critical: Mix immediately and completely to avoid incomplete precipitation.

- (7.) Precipitate on ice for 5 min.

- (8.) Collect the histone pellet at $15\,000 \times g$ for 5 min at 4 °C. Discard supernatant.

Note: TCA precipitation preserves most PTMs. Process pellets within an hour for best downstream recovery.

- (9.) Carefully add 100 μ L cold (–20 °C) 99.9% acetone with HCl to the pellet. Do not disturb the pellet!

This is why: Hydrochloric acid displaces residual TCA, which can interfere with downstream MS applications.

Quality assurance: Since acetone can degrade some plastics, perform washes quickly in compatible tubes.

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(10.) Centrifuge at $15\,000 \times g$ for 2 min at $4\text{ }^{\circ}\text{C}$. Remove acetone using a glass pipette.

Quality assurance: Do not scrape the walls. Leave residual solvent rather than risk losing pellet material. A small amount of acetone will evaporate quickly.

(11.) Repeat acetone wash using $100\ \mu\text{L}$ pure acetone (without HCl).

(12.) Air dry the pellet at room temperature.

Critical: Avoid overdrying. Desiccated pellets may be difficult to redissolve.

Storage of isolated histones

(1.) Store the histone pellet at room temperature for up to one week. For long-term storage, keep at $-80\text{ }^{\circ}\text{C}$.

Hint: Acid-extracted histones are stable for months if kept dry and protected from moisture. For long-term stability and consistent reconstitution, however, it is preferred to fractionate the histones by HPLC and store the purified aliquots as freeze-dried pellets or in low salt buffer. Avoid repeated freeze-thaw cycles once reconstituted.

Analyses

○ Fractionate the histones by HPLC for long-term storage or mass spectrometry analysis.

- Resuspend the dried histone pellet in $85\ \mu\text{L}$ HPLC solvent A.

This is why: Some pellets may not fully dissolve, particularly if total cell lysates were used instead of purified nuclei. Histones are highly soluble, so incomplete dissolution often reflects contamination with insoluble debris. Clarify by centrifugation.

- Inject the sample into a reverse-phase HPLC system equipped with a C18 or C3 column.

Hint: Use columns with large pore size ($300\ \text{\AA}$) and small particle size ($3\text{--}5\ \mu\text{m}$) to preserve histone integrity. For example, Grace™ Vydac® 218TP C18, $2.1\ \text{mm} \times 150\ \text{mm}$, $300\ \text{\AA}$, $3\ \mu\text{m}$.

- Separate histones using a linear gradient of HPLC solvent B, for example, 5–40% B over 30–60 min.

Hint: Histone variants elute between 15–45% B depending on sequence and PTMs. Highly hydrophobic species, like linker histone H1, may elute at 45–60% B.

○ Analyze extracts (or HPLC fractions) by SDS-PAGE and Western blot or prepare for downstream use such as immunoprecipitation or mass spectrometry.

Critical: Ensure buffer exchange or neutralization of residual acid before enzymatic assays or antibody-based detection.

Recipes

Hydrochloric acid (HCl), 0.2 M

| Amount | Ingredient | Stock | Final |
|----------|---------------------------------|-------|-------|
| 2 mL | Hydrochloric acid (HCl), dilute | 1 M | 0.2 M |
| To 10 mL | Water, reagent-grade | | |

0.2 M Hydrochloric acid (HCl)



DANGER

Skin corrosion; Serious eye damage

Date: Sign:

Acetone, with HCl, 99.9%

| Amount | Ingredient | | Stock | Final |
|------------|------------------------|-------------|-------|-------|
| 10 μ L | HCl, 37% | [7647-01-0] | 11.6M | 0.1% |
| To 10 mL | Acetone, reagent-grade | | | |

99.9% Acetone

with HCl



DANGER

Highly flammable liquid and vapor; Serious eye irritation; Drowsiness or dizziness upon single exposure

Date:

Sign:

List of references

M.V. Holt, T. Wang, and N.L. Young, *Curr. Protocol.* **1**(2), e26 (2021).

A. Kossel, *Hoppe-Seyler's Z. Physiol. Chem.* **8**(6), 511–515 (1884).

Change log

2023-10-26 Benjamin C. Buchmuller Adaptation as SOP.

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