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ab221031

Histone Extraction Kit – Rapid / Ultra-pure

For the extraction of histones from mammalian cells and tissue.

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

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1. Overview

ab221031 Histone Extraction Kit - Rapid / Ultra-pure enables the extraction of core histones from mammalian cells or tissues in 1 hour using a simple procedure with 20 minutes' hands-on time. As post-translational modifications, such as acetylation, methylation and phosphorylation are maintained, histones extracted using this kit can be used in a wide variety of downstream applications.

This kit is compatible with three distinct protocols: Standard, Rapid and Ultra-pure Histone Extraction. The Standard Extraction protocol is a 1 hour procedure (of which 20 minutes is hands-on) that is suitable for histone extraction from a wide range of cell lines and tissues. The Rapid Extraction protocol can be used to prepare a crude histone extract quickly for use in applications where histone purity is not critical. It is also recommended for histone extraction using fragile cell lines or in instances where extraction of cytoplasmic histones is desirable. This protocol takes 45 minutes and requires 15 minutes of hands-on time. The Ultra-pure Histone Extraction protocol is a 75-minute procedure (with 25 minutes hands-on time) that yields very pure histones from a wide range of cell lines and tissues.

Each kit contains reagents to perform 100 extractions, where each extraction requires 2×10^6 cells or 20 mg of tissue starting material. Typical yields are around 80 μg of histones from 2×10^6 HeLa cells.

For typical data that can be obtained using this kit please refer to Section 15.

2. Protocol Summary – Standard Extraction

Prepare cell suspension / homogenize tissue



Treat cells with pre-extraction buffer, spin down and discard supernatant



Incubate cells with extraction buffer, spin down and discard pellet



Neutralize supernatant (histone extract)

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

4. Storage and Stability

Store kit as given in the table upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature
Pre-extraction buffer	22 mL	-20°C
Pre-extraction supplement	5.5 mL	-20°C
Histone extraction buffer	22 mL	-20°C
Neutralization buffer	2.2 mL	-20°C
200X DTT	0.3 mL	-20°C
200X Protease inhibitor cocktail	0.15 mL	-20°C

7. Materials Required, Not Supplied

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

- Phosphate-buffered saline (PBS)
- Optional - Trypsin/EDTA solution (if using adherent cells)
- Gels, buffers and equipment for SDS-PAGE
- Dounce homogenizer (if using tissue)
- Reagents or equipment to measure protein concentration e.g. BCA protein assay kit reducing agent compatible ab207003
- Optional - sonicator
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions

8. Technical Hints

- **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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Reagent volumes in the protocols below are provided per extraction (2×10^6 cells or 20 mg of tissue). Scale the volumes up if performing a large-scale extraction.
- If using the kit for the first time with a given cell line or tissue, we recommend analyzing the fractions produced during the protocol by SDS-PAGE (steps marked 'Optional') in order to check the quality of the extraction. If required, Western blotting may be used to confirm the distribution of specific histones between fractions produced during the extraction procedure.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
- Ensure that PBS is at 4°C before starting the experiment and store on ice until required. If the starting material is adherent cells, keep some PBS at room temperature for washing the cells while they are still adhered to the plate.
- Cool the benchtop microcentrifuge to 4°C before starting the experiment.
- If the histone extracts will be used in enzyme activity assays or other downstream applications that are influenced by protease inhibitors, do not add protease inhibitor cocktail to any buffers or fractions.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Pre-extraction buffer:

Ready to use as supplied. Store at -20°C.

9.2 Pre-extraction supplement:

Ready to use as supplied. Store at -20°C.

9.3 Histone extraction buffer:

Ready to use as supplied. Store at -20°C.

9.4 Neutralization buffer:

Ready to use as supplied. Store at -20°C.

9.5 200X DTT:

Ready to use as supplied. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C.

9.6 200X Protease Inhibitor Cocktail:

Ready to use as supplied. Aliquot so that you have enough to perform the desired number of assays. Store at -20°C.

9.7 Buffer preparation

Just before processing cells or tissue prepare buffers as per steps 11.1, 12.1, or 13.1 depending on the extraction protocol chosen.

10. Preparation of starting material

10.1 From adherent cells:

10.1.1 Grow cells to 70-80% confluency on a culture plate or flask (about $2-5 \times 10^6$ cells for a 100 mm plate). Remove the growth medium. Wash the cells twice with room temperature PBS then remove PBS.

10.1.2 Add 1 mL of room temperature PBS per 20 cm² area of cells (e.g. add 3 mL of PBS to a 100 mm plate) and scrape cells into a 15 mL tube.

Alternatively, dispense enough trypsin/EDTA solution to completely cover the monolayer of cells and incubate in a

37°C incubator for about 2 minutes or until cells detach from the surface. When the trypsinization process is complete, the cells will be in suspension and appear rounded. Add serum or media containing serum to the cell suspension as soon as possible to protect cells from damage caused by tryptic activity.

Δ Note: trypsinization may have an impact on the cellular pathway of interest.

- 10.1.3 Count cells using a hemocytometer.
- 10.1.4 Centrifuge the cells for 5 minutes at 200 g and 4°C and discard the supernatant.
- 10.1.5 Wash cell pellet once with ice-cold PBS and resuspend the cells in 10 mL ice-cold PBS.
- 10.1.6 Transfer 2×10^6 cells into a clean tube.
- 10.1.7 Centrifuge for 5 minutes at 200 g and 4°C. Higher speed may be required for some cell types.
- 10.1.8 Discard the supernatant.
- 10.1.9 Cell pellet is ready for processing, proceed to histone extraction protocol of choice: see Section 11 for Standard Extraction, Section 12 for Rapid Extraction or Section 13 for Ultra-pure Histone Extraction.

10.2 From suspension cells:

- 10.2.1 Grow cells to 2×10^6 cells/mL density and collect the cells in a 15 mL tube.
- 10.2.2 Centrifuge the cells for 5 minutes at 200 g and 4°C and discard the supernatant.
- 10.2.3 Wash cell pellet once with ice-cold PBS and resuspend cells in 10 mL of ice-cold PBS.
- 10.2.4 Transfer 2×10^6 cells into a clean tube.
- 10.2.5 Centrifuge for 5 minutes at 200 g and 4°C. Higher speed may be required for some cell types.
- 10.2.6 Discard the supernatant.
- 10.2.7 Cell pellet is ready for processing, proceed to histone extraction protocol of choice: see Section 11 for Standard Extraction, Section 12 for Rapid Extraction or Section 13 for Ultra-pure Histone Extraction.

10.3 From tissues:

This protocol is suitable for both fresh and frozen tissues.

Δ Note: complete sample homogenization is critical for efficient histone extraction.

- 10.3.1 Weigh 20 mg of tissue and cut it into small pieces (approximately 1 mm³) to facilitate homogenization.
- 10.3.2 Wash the tissue twice with ice-cold PBS.
- 10.3.3 Discard the supernatant. The tissue is ready for processing, proceed to histone extraction protocol of choice: see Section 11 for Standard Extraction, Section 12 for Rapid Extraction or Section 13 for Ultra-pure Histone Extraction.

11. Assay Procedure – Standard Extraction

This is a 1 hour procedure (of which 20 minutes is hands-on) that is suitable for histone extraction from a wide range of cell lines and tissues.

11.1 Buffer Preparation (for 2×10^6 cells or 20 mg of tissue):

Δ Note: if the histone extracts will be used in enzyme activity assays or other downstream applications that are influenced by protease inhibitors, do not add protease inhibitor cocktail to the buffers.

- 11.1.1 Transfer 200 μ L of Pre-extraction buffer into a clean 1.5 mL microcentrifuge tubes. Add 1 μ L of 200X protease inhibitor cocktail and 1 μ L of 200X DTT and mix well. Store on ice.
- 11.1.2 Transfer 200 μ L of Extraction buffer into a clean 1.5 mL microcentrifuge tubes. Add 1 μ L of 200X protease inhibitor cocktail and 1 μ L of 200X DTT and mix well. Store on ice.

11.2 Histone Extraction:

- 11.2.1 Resuspend cells pellet or tissue in 200 μ L Pre-extraction buffer with protease inhibitor cocktail and DTT. In case of tissue, use clean, chilled Dounce homogenizer and disrupt tissue with 30 strokes.
Δ Note: View the sample under a microscope to check that the tissue has been homogenized and that most cells have been released into solution. If required, apply more strokes to complete homogenization. Alternatively, pass the sample through appropriate sieve(s) to remove fragments resistant to homogenization. Sieving may result in sample misrepresentation.
- 11.2.2 Incubate the suspension on ice for 10 minutes.
- 11.2.3 Centrifuge cell suspension or tissue homogenate for 2 minutes at 1,000 g and 4°C.
- 11.2.4 Using a pipette, transfer as much supernatant as possible into a fresh microcentrifuge tube taking care to not disturb the pellet which contains histones.
- 11.2.5 Optional: keep the supernatant on ice for SDS-PAGE or BCA analysis. If the analysis is not required, the supernatant can be discarded.

- 11.2.6 Resuspend the pellet from Step 11.2.4 in 200 μ L Extraction buffer with protease inhibitor cocktail and DTT.
- 11.2.7 Incubate suspension on ice for 30 minutes. At this point histones translocate from the pellet to the soluble fraction.
- 11.2.8 Centrifuge suspension for 2 minutes at 10,000 g and 4°C.
- 11.2.9 Using a pipette, transfer 200 μ L of the supernatant into a fresh microcentrifuge tube, taking care to not disturb the pellet. Keep both supernatant and pellet on ice. The supernatant contains the histones while the pellet contains other proteins and genomic DNA.
- 11.2.10 Optional: using a pipette, gently remove and discard any remaining supernatant from the pellet. Keep the pellet for SDS-PAGE or BCA analysis. If the analysis is not required, the pellet can be discarded.
- 11.2.11 Add 20 μ L of neutralization buffer to the supernatant from step 11.2.9 and mix well to obtain histones extract with neutral pH. Use the histones extract in the application of interest immediately or aliquot and freeze at -80°C.

12. Assay Procedure – Rapid Extraction

This is a 45 minute protocol (15 minutes' hands-on time) that can be used to prepare a crude histone extract quickly for use in applications where histone purity is not critical. It is also recommended for histone extraction using fragile cell lines or in instances where extraction of cytoplasmic histones is desirable.

12.1 Buffer Preparation (for 2×10^6 cells or 20 mg of tissue):

Δ Note: if the histone extracts will be used in enzyme activity assays or other downstream applications that are influenced by protease inhibitors, do not add protease inhibitor cocktail to the buffers.

- 12.1.1 Transfer 40 μL of Pre-extraction buffer into a clean 1.5 mL microcentrifuge tubes. Add 160 μL of Extraction buffer, 1 μL of 200X protease inhibitor cocktail and 1 μL of 200X DTT and mix well. This Extraction cocktail will be used in the following steps.
- 12.1.2 Store on ice.

12.2 Histone Extraction:

- 12.2.1 Resuspend cells pellet or tissue in 200 μL Extraction cocktail with protease inhibitor cocktail and DTT. In case of tissue, use clean, chilled Dounce homogenizer and disrupt tissue with 30 strokes.

Δ Note: View the sample under a microscope to check that the tissue has been homogenized and that most cells have been released into solution. If required, apply more strokes to complete homogenization. Alternatively, pass the sample through appropriate sieve(s) to remove fragments resistant to homogenization. Sieving may result in sample misrepresentation.
- 12.2.2 Incubate the suspension on ice for 30 minutes.
- 12.2.3 Centrifuge cell suspension or tissue homogenate for 2 minutes at 1,000 g and 4°C.
- 12.2.4 Using a pipette, transfer as much supernatant as possible into a clean microcentrifuge tube, taking care to not disturb the pellet. Keep both supernatant and pellet on ice. The supernatant contains the histones and the pellet contains other proteins and genomic DNA.

- 12.2.5 Optional: using a pipette, gently remove and discard any remaining supernatant from the pellet. Keep the pellet for SDS-PAGE or BCA analysis. If the analysis is not required, the pellet can be discarded.
- 12.2.6 Add 16 μ L of neutralization buffer to the supernatant from Step 12.2.4 and mix well to obtain histones extract with neutral pH. Use the histones extract in the application of interest immediately or aliquot and freeze at -80°C .

13. Assay Procedure – Ultra-pure Histone Extraction

This is a 75 minutes procedure (25 minutes hands-on time) that yields very pure histones extract. When using this protocol to extract histones from a given cell line or tissue for the first time, we recommend titration as described below to determine the optimal ratio of Pre-extraction buffer to Pre-extraction supplement for extraction from that cell line or tissue.

A western blot should be performed after extraction using an anti-H3 or anti-H2B antibody to determine which ratio of Pre-extraction buffer to Pre-extraction supplement gives the strongest signal.

13.1 Buffer Preparation (for 2x10⁶ cells or 20 mg of tissue):

Δ Note: if the histone extracts will be used in enzyme activity assays or other downstream applications that are influenced by protease inhibitors, do not add protease inhibitor cocktail to the buffers.

- 13.1.1 Transfer 200 μ L of Pre-extraction buffer into a clean 1.5 mL microcentrifuge tubes. Add 1 μ L of 200X protease inhibitor cocktail and 1 μ L of 200X DTT and mix well. Store on ice.
- 13.1.2 If using this protocol with a cell line or a tissue for the first time, optimization of the histone extraction buffers is required. For optimization, prepare 6 x 1.5 mL microcentrifuge tubes of Pre-Extraction Buffer + Supplement as described below and store on ice.

Tube #	Pre-extraction buffer (μ L)	Pre-extraction supplement (μ L)	200X Protease Inhibitor Cocktail (μ L)	200X DTT (μ L)
1	200	0	1	1
2	192	8	1	1
3	184	16	1	1
4	176	24	1	1
5	168	32	1	1
6	160	40		

Δ Note: if optimal conditions have already been established, prepare only one combination of Pre-Extraction Buffer and Supplement, remember to include 1 μ L of 200X protease inhibitor cocktail and 1 μ L of 200X DTT.

- 13.1.3 For each extraction, transfer 200 μ L of Extraction Buffer, 1 μ L 200X protease inhibitor cocktail and 1 μ L 200X DTT into clean 1.5 mL microcentrifuge tubes.
- 13.1.4 Store on ice.

13.2 Histone Extraction:

- 13.2.1 Resuspend cells pellet or tissue in 200 μ L Pre-extraction buffer with protease inhibitor cocktail and DTT. In case of tissue, use clean, chilled Dounce homogenizer and disrupt tissue with 30 strokes.
 Δ Note: View the sample under a microscope to check that the tissue has been homogenized and that most cells have been released into solution. If required, apply more strokes to complete homogenization. Alternatively, pass the sample through appropriate sieve(s) to remove fragments resistant to homogenization. Sieving may result in sample misrepresentation.
- 13.2.2 Incubate the suspension on ice for 10 minutes.
- 13.2.3 Centrifuge cell suspension for 2 minutes at 1000 g and 4°C.
- 13.2.4 Using a pipette, transfer as much supernatant as possible into a clean microcentrifuge tube, taking care to not disturb the pellet which contains histones.
- 13.2.5 Optional: keep the supernatant on ice for SDS-PAGE or BCA analysis. If the analysis is not required, the supernatant can be discarded.
- 13.2.6 Resuspend the pellet from Step 13.2.4 in 200 μ L of Pre-Extraction buffer + **Pre-Extraction supplement** with protease inhibitor cocktail and DTT.
- 13.2.7 Incubate suspension on ice for 10 minutes.
- 13.2.8 Centrifuge suspension for 2 minutes at 10,000 g and 4°C.
- 13.2.9 Using a pipette, transfer as much supernatant as possible into a clean microcentrifuge tube, taking care not to disturb the pellet which contains histones.
- 13.2.10 Optional: keep the supernatant on ice for SDS-PAGE or BCA analysis. If the analysis is not required, the supernatant can be discarded.
- 13.2.11 Resuspend the pellet from Step 13.2.9 in 200 μ L Extraction buffer with protease inhibitor cocktail and DTT.
- 13.2.12 Incubate suspension on ice for 30 minutes. At this point histones translocate from the pellet to the soluble fraction.

- 13.2.13 Centrifuge suspension for 2 minutes at 10,000 g and 4°C.
- 13.2.14 Using a pipette transfer 200 µL of the supernatant into a fresh microcentrifuge tube, taking care to not disturb the pellet. Keep both supernatant and pellet on ice. The supernatant contains the histones while the pellet contains other proteins and genomic DNA.
- 13.2.15 Optional: using a pipette, gently remove and discard any remaining supernatant from the pellet. Keep the pellet for SDS-PAGE or BCA analysis. If the analysis is not required, the pellet can be discarded.
- 13.2.16 Add 20 µL of neutralization buffer to the supernatant from Step 13.2.14 and mix well to obtain histones extract with neutral pH. Use the histones extract in the application of interest immediately or aliquot and freeze at -80°C.

14. Protein quantification and analysis:

- 14.1 Measure the concentration of the histone extract. For in-bulk quantification, we recommend using ab207003, BCA protein assay kit compatible with reducing agent, which has been developed for accurate protein quantification in the presence of DTT present in the samples. For more accurate quantification of histones, we recommend running samples on SDS-PAGE in parallel with BSA standards followed by Coomassie Blue staining and densitometer scanning.
- 14.2 Analyze the sample of histone extract by SDS-PAGE followed by Coomassie Blue staining to assess purity.
- 14.3 If additional SDS-PAGE samples were prepared (steps marked Optional), analyze these in parallel to assess the distribution of specific histones between fractions by Western Blot. If more than 5% of total histones are present in the pre-extraction and/or insoluble fractions, refer to Troubleshooting Section.

15. Typical data

Data provided for demonstration purposes only.

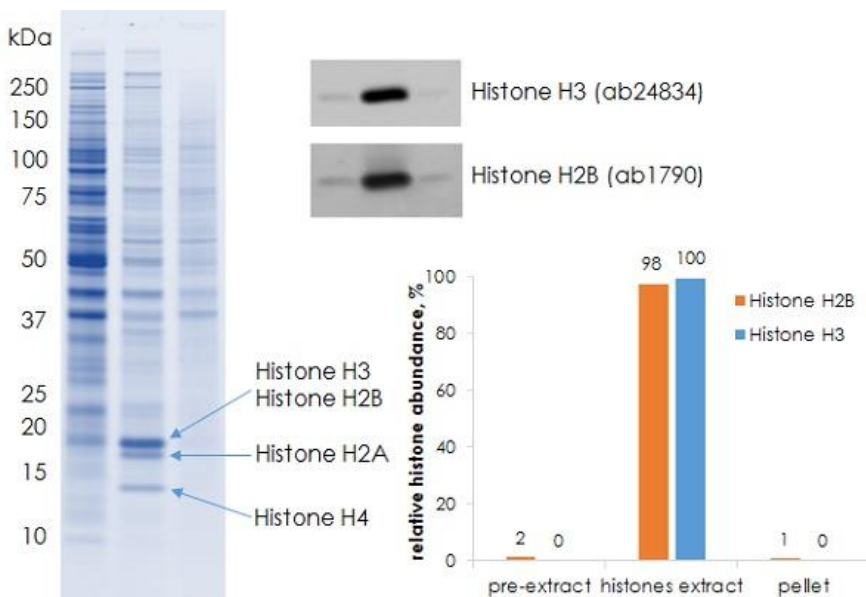


Figure 1: Histone extraction from HeLa cells

2×10^6 of HeLa cells were processed as per the standard protocol. The resulting fractions were analysed by SDS PAGE followed by Coomassie Blue staining (left panel) or Western Blot analysis (right panel). Western Blot was performed using rabbit polyclonal to H2B (ab1790) and mouse monoclonal to H3 (ab24834) primary antibodies and fluorophore-labelled secondary antibodies to assess distribution of specific histones between the fractions (right panel). Lanes: 1. Pre-extract; 2. Histone extract; 3. Pellet.

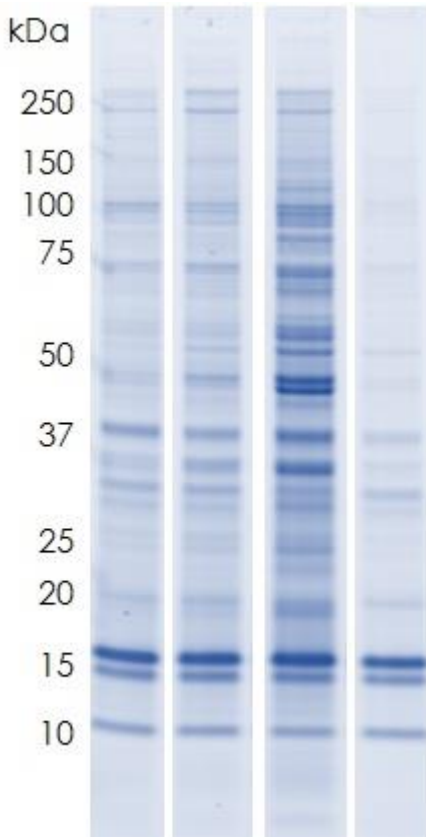


Figure 2: Comparison of different histone extraction methods

2×10^6 of HeLa cells were processed using competitor kit or Abcam ab221031 Histone extract kit according to standard, rapid or ultra-pure protocol. For ultra-pure protocol 176 μ L pre-extraction buffer were combined with 24 μ L of pre-extraction supplement. The resulting histone extract fractions were analysed by SDS PAGE. Lanes: 1. Competitor kit; 2. Standard protocol; 3. Rapid protocol; 4. Ultra-pure protocol.

16. Troubleshooting

Problem	Reason	Solution
No pellet after incubating cells in pre-extraction buffer	Insufficient centrifugation speed. Some cell types may be more difficult to pellet than others.	Increase centrifugation speed.
	Cells completely lysed following incubation in Pre-extraction buffer. Some cell types or treated cells may be particularly prone to lysis upon addition of Pre-extraction buffer, despite its mild formulation. A viscous sample that is difficult to pipette may be indicative of lysis and the subsequent release of genomic DNA into the sample.	Examine samples with a phase contrast microscope. If no cells and/nuclei are visible, repeat extraction using Pre-extraction buffer diluted 2-10-fold with deionised water. Alternatively, try the rapid histone extraction protocol (Section 12).
Low yield of histones	Insufficient amount of starting material.	Double check the number of cells or mg of tissue in input material.
	Type of starting material. Although histones are very abundant proteins in most cells, some cell types, e.g. red blood cells, may have naturally very low histone content.	Use higher amounts of input material per extraction.
	Handling error or unexpected losses of histones in pre-extraction and/or pellet fractions.	Check the distribution of histones between fractions by SDS-PAGE. If over 5% of histones are found in either the pre-extraction fraction or the pellet, please refer to the relevant sections of this Troubleshooting guide.
Higher concentration of histones is required for downstream applications		Reduce volumes of Extraction buffer and Neutralization buffer. Use 40 μ L of extraction buffer in step 11.2.6 and 4 μ L of neutralization buffer in step 11.2.11 of the standard protocol. Note that this may reduce total amount of histones recovered although the final concentration will be higher.

	<p>As histones are relatively small, highly positively charged proteins, it is important to choose the ultrafiltration unit carefully. When establishing ultrafiltration procedure, we recommend checking both flow through and concentrate by SDS-PAGE or other protein quantification method alongside the starting histone extract to monitor losses due to ultrafiltration.</p>	<p>Concentrate your sample by ultrafiltration.</p>
		<p>Concentrate your sample using protein precipitation by trichloroacetic acid (TCA). Do not add neutralization buffer in the final step of the histone extraction section (step 11.2.11 in the standard protocol) prior to TCA precipitation.</p>
Higher histone purity is required		<p>Follow Ultra-pure Histone Extraction protocol</p>
		<p>Precipitate proteins using trichloroacetic acid (TCA). Do not add neutralization buffer in the final step of the histone extraction section (step 11.2.11 in the standard protocol) prior to TCA precipitation. Some of the contaminating proteins will not re-dissolve in water following TCA precipitation and acetone washes while majority of the histones will be soluble.</p>
		<p>Combine both options above</p>
More than 5% of histones are detected in the pre-extraction fraction by SDS-PAGE	<p>Handling error. Any carry over of the cell pellet into the supernatant will result in a significant increase in histones in the pre-extraction fraction as the pellet is enriched in cell nuclei.</p>	<p>Following incubation with Pre-extraction buffer, make sure that supernatant is carefully separated from the pellet</p>

	Cell lysis. Although the Pre-extraction buffer has a very mild formulation, some cell types or treated cells may be prone to lysis upon treatment with Pre-extraction buffer	Repeat extraction using Pre-extraction buffer that has been diluted 2-10 fold with deionized water. Alternatively, follow rapid histone extraction protocol (Section 12).
More than 5% of histones are detected in the pellet fraction by SDS-PAGE	Handling error	Following incubation with Extraction buffer, ensure that the supernatant is completely separated from the pellet before the pellet is solubilized
	With tissues, complete sample homogenization in Dounce homogenizer is of paramount importance for efficient histone extraction	Use a microscope to check that the tissue is homogenized and most cells/nuclei are released into solution. If required, apply more strokes complete homogenization. Alternatively pass the sample through appropriate sieve(s) to remove fragments resistant to homogenization. Sieving may result in sample misrepresentation

17. Notes

Technical Support

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<https://www.abcam.cn/contact-us> (China)

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