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Acid Extraction of Total Histone from Colon Cancer HCT116 Cells

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[Abstract] Histone acid extraction assay is a popular method to determine histone modification levels in mammalian cells. It includes three steps: first, histones are released from chromatin by sulfuric acid; trichloroacetate (TCA) is then added to precipitate histones; and finally, histones are dissolved in double-distilled H_2O (ddH_2O). Here we present a detailed histone acid extraction assay in our laboratory using a colon cancer cell line, HCT116, as a model.

[Background] The nucleosome is the fundamental unit of eukaryotic chromatin, which is composed of a histone octamer (2 copies of H3, H4, H2A, H2B, respectively) wrapping by DNA (Strahl and Allis, 2000). The amino terminal of histone is subjected to a variety of post-translational modifications, such as methylation, acetylation, phosphorylation, ubiquitylation and sumoylation (Kouzarides, 2007). Although the function of these modifications has remained elusive, there is ever-growing studies suggest that histone modifications play vital roles in intracellular processes (Bannister and Kouzarides, 2011). Therefore, it is important to extract histones efficiently to detect histone modifications.

Histones can be extracted via different methods, in which histone acid extraction assay is one of the most popular procedures. It does not interrupt post-translational modifications of histones, and so it is very good for histone modification analysis. It has been tested that the extracted histones can be used in Western blot, and maybe other assays (not fully tested). However, immunoprecipitation is not recommended. In this protocol, we will present a detailed histone acid extraction assay, and describe how to release histones from chromatin, how to precipitate histones, and how to wash and dissolve histones in ddH_2O.

Materials and Reagents

1. 6 cm plate
2. 1.5 ml tubes (Corning, Axygen®, catalog number: MCT-150-C)
3. Human colon cancer cell line HCT116 (ATCC)
4. Phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Gibco™)
5. Acetone
6. Double-distilled H_2O (ddH_2O)
7. 2x SDS loading buffer (containing 200 mmol/L DTT)
8. Tris-HCl (pH = 8.0)

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9. Potassium chloride (KCl)
10. Magnesium chloride ($MgCl_2$)
11. Dithiothreitol (DTT)
12. Protease inhibitor cocktail (Sigma-Aldrich, catalog number: P8340)
13. Sulfuric acid
14. Trichloroacetate (TCA) (Sigma-Aldrich, catalog number: T6399)
15. Trichloroacetic acid (TCA) solution (see Recipes)
16. Lysis buffer(see Recipes)

Equipment

1. Pipettor (Eppendorf)
2. Centrifuge (cooled and room-temperature)(Eppendorf)
3. Rotator
4. Spectrophotometer (Bio-Rad Laboratories)
5. Metal bath

Procedure

An overview of the whole procedure is schematized in Figure 1.

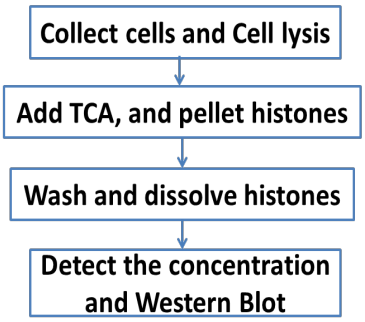

Figure 1. Scheme of the complete procedure described in the protocol

1. Collect HCT116 cells (6 cm plate, 70-90% confluent) using any commonly used method in 1 ml ice-cold PBS and pellet (1,000 *x g*, 5 min, 4 °C).
2. Discard the supernatant, add 1 ml ice-cold PBS and pipet gently to resuspend the cells, then pellet it again (1,000 *x g*, 5 min, 4 °C).
3. Discard the supernatant, resuspend cell pellet in 400 µl lysis buffer, and incubate for 30 min with rotating at 4 °C.
4. Pellet the intact nuclei by spinning in cooled centrifuge (12,000 *x g*, 10 min, 4 °C).
5. Transfer the supernatant containing histones into a fresh 1.5 ml tube.

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6. Add 133 µl TCA drop by drop to histone solution and invert the tube several times to mix the solutions (final concentration of TCA is 25%). (It is better to add one drop and invert the tube at once to mix the solutions, and then add another drop.) The solution will appear milky over time.
7. Incubate on ice for 30 min or overnight.
8. Pellet histones by spinning in cooled centrifuge (12,000 *x g*, 10min, 4 °C).
9. Carefully remove supernatant and wash histone pellet with 1 ml ice cold acetone without disturbing it. Acetone is used to remove acid from the solution without dissolving the histone pellet.
10. Spin down in centrifuge at 12,000 *x g*, 10 min at 4 °C.
11. Repeat steps 9 and 10 to wash histone pellet again.
12. Carefully remove all of the supernatant and air-dry histone pellet for 30 min at room temperature.
13. Dissolve histone pellet in appropriate volume of ddH_2O (for example, 100 µl is appropriate for a 6 cm plate), and transfer into fresh 1.5 ml tube.
14. Detect the concentration of histone solution with the spectrophotometer (using OD 280), and we may acquire 1-2 µg histone protein from a 70-90% confluent well on a 6 cm plate.
15. Add the same volume of 2x SDS loading buffer into histone solution, and boil it on the metal bath (100 °C, 5 min), then subject to Western blot.

Representative data

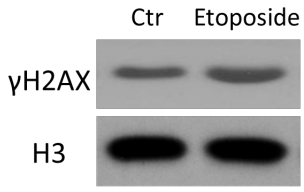

Figure 2. HCT116 cells were treated with etoposide and subjected to histone acid extraction. Immunoblotting was performed with the indicated antibodies.

Note: Relative Western blots have also been published in our paper Oncogene (Cao et al., 2016). For details, please refer to Figure 4 in Cao et al., 2016 (https://www.researchgate.net/publication/274316705_ATM-mediated_KDM2A_phosphorylation_is_required_for_the_DNA_damage_repair).

Recipes

1. Trichloroacetic acid (TCA) solution
   500 g TCA in 227 ml ddH_2O
   Store at room temperature, and protect from light
2. Lysis buffer
   10 mM Tris-HCl, pH 8.0
   1 mM KCl

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1.5 mM $MgCl_2$
10 mM DTT (added immediately before use)
1x protease inhibitor cocktail (added immediately before use)
0.4 M sulfuric acid (added immediately before use)

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