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We use this protocol and it's working

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Histone extraction HLB protocol

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ABSTRACT

Protocol to extract histone proteins using a hypotonic lysis buffer for isolation of nuclei prior to acid extraction of histones that is easily amenable to label-free MS owing to the lack of detergents in the lysis buffer.

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Isolate nuclei

1 Start from a dry (snap-frozen) cell pellet

2 Add hypotonic lysis buffer (HLB) to cell pellet: **200 µL for 1x10^6 cells** and resuspend softly

> **Note**
>
> HLB buffer: 10 mM Tris-HCl pH 8.0, 1 mM KCl, 1.5 mM MgCl2 supplemented with 1 mM DTT, 1 mM PMSF, Halt Protease and Phosphatase Inhibitor Cocktail 100x (78440) and phosphatase inhibitor cocktails II and III (P5726 and P0044, Sigma-Aldrich, 1 mL of cocktail for 100 mL of buffer)

3 Rotate for 30 min at 4°C to promote lysis of cell membrane (mechanical shear)

4 Pellet the nuclei by centrifugation at 10.000g for 10 min at 4°C

5 Discard the supernatant

Extract histones

6 Resuspend the pellet in 0.4N hydochloric acid (HCl) by soft pipetting until no clumps left in solution by adding **125 µL HCl for 1x10^6 cells** (if necessary: vortex)

7 Incubate for **30 min** in acid on rotator at 4°C to promote lysis of nuclei and solubilization of histones

8 Spin down for 10 min at 4°C and 16.000 g

9 Transfer supernatant to new Eppendorf (histones are present in the acid since they are alkaline proteins)

Isolate histones

10 Add, drop by drop, trichloroacetic acid (TCA) until a final concentration of 33% is reached to promote precipitation of histones and invert the tube several times (results in a milky solution)

11 Incubate on ice for 30min

12 Spin for 10 min at 4°C and 16.000 g to pellet the histones

13 Remove the supernatant

Note

Be careful: the pellet is not always visible

Wash steps

14 Add ice-cold acetone (do not resuspend the pellet) to remove TCA, make sure the pellet is fully covered with acetone

15 Spin for 5 min at 4°C and 16000 g

16 Remove the supernatant

17 Add cold acetone again (do not resuspend the pellet) to remove TCA

18 Spin for 5 min at 4°C and 16000 g

19 Remove the supernatant

20 Dry at room temperature for 30 min (until no acetone left)

Note

Samples can be stored at -80°C or -20°C until further use (propionylation/digestion) or part of the sample can be prepared to perform sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Preparation for SDS-PAGE

21 Resuspend in MilliQ water (50 µl for $1x10^6$ cells)

22 Transfer 400.000 cells to a new Eppendorf tube for gel-electrophoresis (optionally)

Note

If there are still clumps left: Spin for 10 min at 4°C and 16000 g and transfer the supernatant in a fresh Eppendorf

23 Vacuum dry the samples (SpeedVac)

Note

Store both Eppendorf tubes (for propionylation/digestion and SDS-PAGE) at -20°C or -80°C until further use