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Protocol status: Working 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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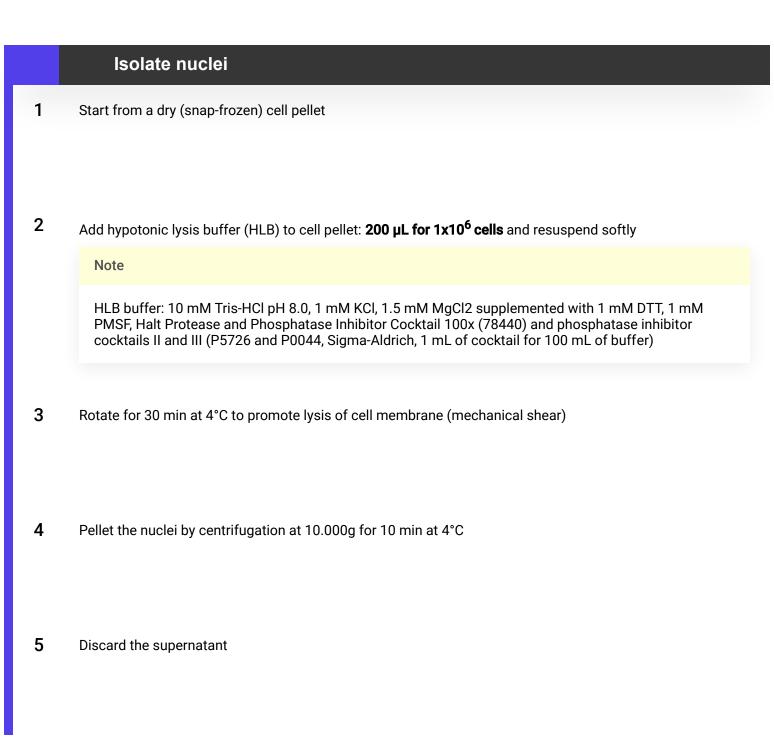
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Extract histones

- Resuspend the pellet in 0.4N hydochloric acid (HCl) by soft pipetting until no clumps left in solution by adding 125 µL HCl for 1x10⁶ 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
- 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

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

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13 Remove the supernatant



Note

Be careful: the pellet is not always visible

Wash steps

- 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

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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 1x10⁶ cells)

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 $^{\circ}$ C or -80 $^{\circ}$ C until further use