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Oirect acid extraction

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Protocol status: Working We use this protocol and it's working

Created: Jan 31, 2024

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ABSTRACT

Protocol to extract histones from cells using direct acid extraction with the purpose of label-free LC-MS/MS measurement.

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Last Modified: Jan 31, 2024 MATERIALS

PROTOCOL integer ID: 94448

Protein lobind Eppendorf tubes

Thermoshaker

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Cooled centrifuge (4°C)

BOF

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Ice

MilliQ water

12 M HCL (dilute to 0.4N HCl: for 300 mL: 10 mL 12M (37%) stock + 290 mL milliQ water)

TCA powder (make 100% TCA solution)

Ice-cold acetone

1 Resuspend the pellet in 0,4 N HCl by soft pipetting until no clumps are left in solution (125 μL for 1x10⁶cells)

Note

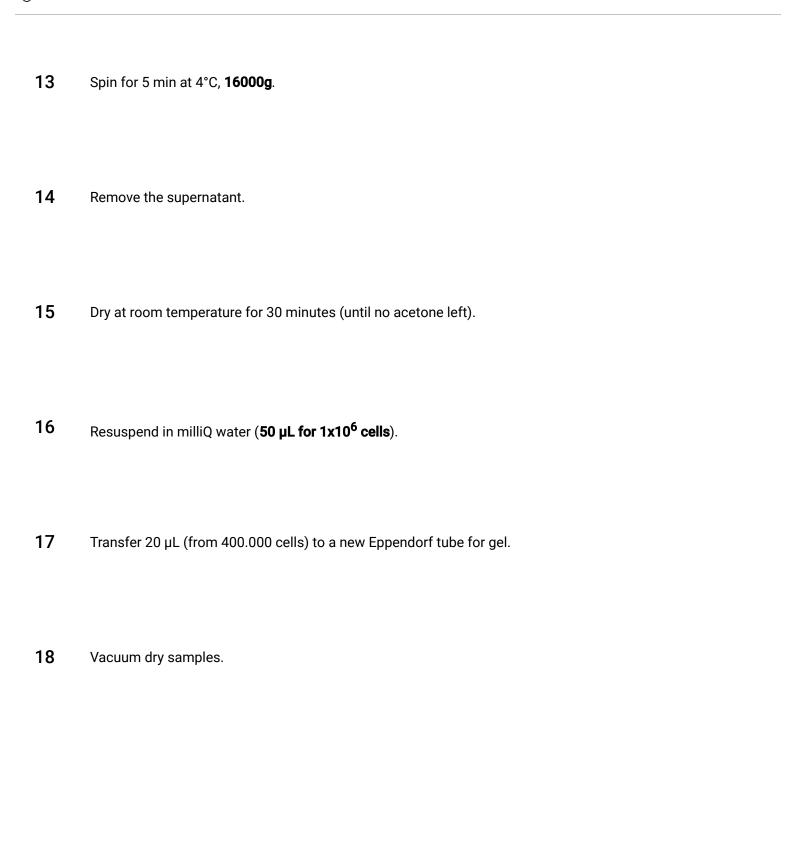
If necessary, vortex softly

- 2 Incubate **4h** in acid on rotator **4°C** to promote lysis of nuclei and solubilization of histones.
- 3 Spin for 10 minutes at **4°C, 16000 g**.
- 4 Transfer supernatant to new Eppendorfs.

Note

Histones are present in the acid since they are alkaline proteins

5 Add, drop by drop (very slowly and in the middle of the eppendorf), TCA (**final concentration 33%: 61.25μl for 1x10⁶)** to promote precipitation of histones and invert the tube several times.



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