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

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Direct acid extraction

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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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MATERIALS

Protein lobind Eppendorf tubes
Thermoshaker
Cooled centrifuge (4°C)
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 1x10^{6}cells**)

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 1x10^6)** to promote precipitation of histones and invert the tube several times.

6 Incubate on ice for 30 minutes.

7 Spin for 10 min at 4°C, **16000g** to pellet the histones.

Note

Important: Place all the eppendorfs in the same position => makes it easier to know where the pellet is localized

8 Remove the supernatant.

Note

Be careful, the pellet is not always visible, smear on wall.

9 Add ±150 µL ice-cold acetone (don't resuspend the pellet) to remove TCA.

10 Spin for 5 min at 4°C, **16000g**.

11 Remove the supernatant.

12 Add ±150 µL cold acetone (don't resuspend the pellet) to remove TCA. If not all the acid is removed it will destroy the SDS-gel.

13 Spin for 5 min at 4°C, **16000g**.

14 Remove the supernatant.

15 Dry at room temperature for 30 minutes (until no acetone left).

16 Resuspend in milliQ water (**50 µL for 1x10^{6} cells**).

17 Transfer 20 µL (from 400.000 cells) to a new Eppendorf tube for gel.

18 Vacuum dry samples.