Jan 31, 2024

Direct acid extraction

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6×6odlqe/v1

Sigrid Verhelst[1], Laura Corveleyn[1]

[1]ProGenTomics, Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium

Metabolomics Protocols & Workflows
Tech. support email: **bbmisraccb@gmail.com**

Laura Corveleyn
ProGenTomics, Laboratory of Pharmaceutical Biotechnology, Gh...

DOI: https://dx.doi.org/10.17504/protocols.io.bp2l6x6odlqe/v1

Protocol Citation: Sigrid Verhelst, Laura Corveleyn 2024. Direct acid extraction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6x6odlqe/v1

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

Created: January 31, 2024

Last Modified: January 31, 2024

Protocol Integer ID: 94448

Keywords: histones from cell, using direct acid extraction, direct acid extraction with the purpose, direct acid extraction protocol, histone, extraction, label free lc

Funders Acknowledgements:
BOF
Grant ID: Mandate 01D23313

Disclaimer

Abstract

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

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 1×10^{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 1×10^6)** 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 1×10^6 cells**).

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

18 Vacuum dry samples.

Protocol references

Govaert E, Van Steendam K, Scheerlinck E, Vossaert L, Meert P, Stella M, Willems S, De Clerck L, Dhaenens M, Deforce D. Extracting histones for the specific purpose of label-free MS. Proteomics. 2016 Dec;16(23):2937-2944. doi: 10.1002/pmic.201600341. PMID: 27718312; PMCID: PMC5157773.