BIORUPTOR® PROTOCOL

WESTERN BLOT - PROCEDURE FOR SIMULTANEOUS EXTRACTION OF CYTOPLASMIC, NUCLEAR AND CHROMATIN PROTEINS WITH BIORUPTOR®

The regular protocol for the extraction of histone requires an acid extraction, making impossible the detection of any other cytoplasmic and nuclear proteins from the same extract. Using Bioruptor®, Slimane AIT-SI-ALI and his "epigenetic and cell fate" team from the University Paris Descartes developed a protocol allowing the simultaneous extraction of histone and other proteins.

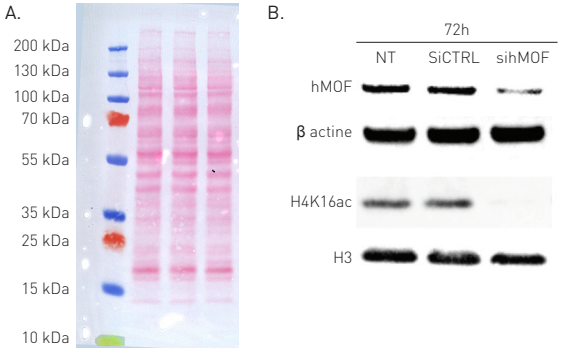

Figure: Protein staining of cell lysates (containing both chromatin and soluble proteins) obtained with the protocol developed by Lauriane Fritsch form the laboratory of Slimane AIT-SI-ALI. A) Ponceau S Staining Western Blot of Hep G2 cells lysates obtained using the Bioruptor®. The rectangle indicates the location of histones. B) Western Blot of Hep G2 cells transfected and cultivated for 72h with a siRNA against hMOF, a histone H4 K16-specific acetyl transferase. Both soluble (hMOf and B-actine) and chromatin (Histone 3 and 4) proteins are obtained on the same cell extract.

1. Material required and buffer preparation

- Bioruptor® Plus (Diagenode, Cat. No. B01020001) with 15 ml tube holder and 15 mL sonication probes (Diagenode, Cat. No. B01200013) or 1.5 ml tube holder
- 15mL TPX tubes (Diagenode, Cat. No. C30010009) or 1.5 ml TPX tubes (Diagenode, Cat. No. C30010010)
- PBS
- Mnase (Sigma-Aldrich, Cat. No. n3755). Reconstitute in ultra pure water at 0.5U/µl.
- 0.1 M CaCl2
- RIPA buffer: commercially available or homemade buffer
  50mM tris pH 7,5
  150 mM NaCl
  1% NP40
  0,5% Na-deoxycholate
  0,1% SDS

2. Procedure

- Wash cells with PBS and pellet cells by centrifugation in 15 ml TPX tubes or 1,5 ml TPX tubes (depending on the volume: link to the Diagenode tube guide).
- Add 3x the volume of ice-cold RIPA buffer.
- Incubate 10 minutes on ice.
- Add reconstitute Mnase to a final concentration of 0.0025U/µl.
- Add CaCl2 to a final concentration of 1 mM.
- Incubate 15 minutes at 37°C.
- Pre-cooled Bioruptor® at 4°C with the water cooling system (Diagenode, Cat. No. B02010002 or B02010003) or use cold distilled water.
- Install samples in Bioruptor® and sonicate at High Power for 8 cycles (sonication cycle: 15 sec ON, 60 sec OFF).
- Centrifuge at 13,200 rpm for 10 minutes at 4°C.
- Collect supernatant and discard pellet.
- Determine protein concentration.
- Store aliquots at -20°C or proceed with the western blot analysis. The use of 10 to 30ug is recommended.

3. Selected publications

1. Mozzetta C, Pontis J, Fritsch L, Robin P, Portoso M, Proux C, et al. The histone H3 lysine 9 methyltransferases G9a and GLP regulate polycomb repressive complex 2-mediated gene silencing. Mol Cell. 2014 Jan 23;53(2):277–89.
2. Guasconi V, Pritchard L-L, Fritsch L, Mesner LD, Francastel C, Harel-Bellan A, et al. Preferential association of irreversibly silenced E2F-target genes with pericentromeric heterochromatin in differentiated muscle cells. Epigenetics. 2010 Nov;5(8):704–9.
3. Fritsch L, Robin P, Mathieu JRR, Souidi M, Hinaux H, Rougeulle C, et al. A subset of the histone H3 lysine 9 methyltransferases Suv39h1, G9a, GLP, and SETDB1 participate in a multimeric complex. Mol Cell. 2010 Jan 15;37(1):46–56.

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