BIORUPTOR® PROTOCOL

MASS-SPECTROMETRY: PROCEDURE FOR SHOTGUN PROTEOMICS

Mass Spectrometry is the preferred method for the sequencing and characterization of proteins. Shotgun proteomics analysis of cell lines and tissues relies on stringent isolation of proteins in addition to degradation and removal of unwanted components like nucleic acids. Therefore, it requires mechanical breakdown accompanied by denaturing agents and heating of the samples. Matthias Mann and his team from the Department of Proteomics and Signal Transduction at the Max Plank Institute of Biochemistry developed and employ highly efficient protocols for protein isolation in conjunction with Bioruptor to perform quantitative proteomics.

1. Material required and buffer preparation

- Bioruptor® Plus (Diagenode, Cat. No. B01020001) with 1.5 ml tube holder
- 1.5 ml Diagenode TPX microtubes (C30010010)
- Thermomixer
- 0.5 M CAA (2-Chloroacetamide)
- Acetone (stored at -20°C)
- Buffer composition:

Denaturing buffer:

Detergent-free buffer:

- 10mM TCEP,
- 40mM CAA (2-Chloroacetamide),
- 100mM Tris pH 8.5
- 1% (w/v) sodium deoxycholate

or

- 6M GdmCl,
- 10mM TCEP
- 40mM CAA,
- 100mM Tris pH 8.5

or

- 8M Urea,
- 10mM TCEP
- 40mM CAA,
- 100mM Tris pH 8.5

Detergent based buffer

- 4% SDS,
- 10 mM Hepes,
- pH 8.0,
- 10 mM DTT

2. Procedure

1. Pre-cool the Bioruptor® at 4°C with the water cooling system (Diagenode, Cat. No. B02010002 or B02010003) or use cold distilled water.
2. Wash cells or tissues with PBS and pellet samples by centrifugation in 1.5 ml tubes (at 4°C).
3. Discard supernatants and add denaturing buffer at a ratio of 1–5 µg protein per 1 µl buffer (as an example S. cerevisiae, S. pombe and HeLa cells contain approximately 3 pg/cell, 9 pg/cell and 200 pg/cell of protein, respectively).
4. Briefly vortex to resuspend samples.
5. Agitate and heat samples for 5 min at 95°C (do not heat up Urea containing buffers !) using a thermomixer (e.g. mixing frequency 1000 rpm).
6. Install 1.5 ml Diagenode tubes (sample volume 100 – 300 µL) in the Bioruptor® tube holder and sonicate at High Power for 10 min (sonication cycle: 15 sec ON, 15 sec OFF).
7. Repeat step 6 if samples are still slurry
8. For detergent based buffers: Purify samples by precipitation (Removal of MS incompatible buffer):
   - a.i. Proceed with 100 ug protein (keep protein concentration above 0.5 ug/ ul)
   - a.ii. Alkylate with 50 mM CAA (2-Chloroacetamide, 0.5 M stock) and incubate for 45 min at RT

a.iii. Precipitate by the addition of 4X volumes of -20°C acetone followed by an overnight incubation

a.iv. Collect proteins by centrifugation for 15 min at 10,000 x g at 4°C

a.v. Wash pellet twice with -20°C 80% acetone

a.vi. Resuspend proteins in MS and digestion compatible buffer (e.g. 8M Urea in 50mM Ammonium Bicarbonate)

9. Adjust to enzyme buffer requirements

10. Proceed with preferred enzymatic digestion

3. Selected publications

1. Humphrey SJ, Azimifar SB, Mann M **High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics**, *Nat Biotechnol. 2015, doi:10.1038/nbt.3327*

2. Schiller HB, Fernandez IE, Burgstaller G, Schaab C, Scheltema RA, Schwarzmayr T, Strom TM, Eickelberg O, Mann M. **Time- and compartment-resolved proteome profiling of the extracellular niche in lung injury and repair**, *Mol Syst Biol. 2015, doi:10.15252/msb.20156123*

3. Hornburg D, Drepper C, Butter F, Meissner F, Sendtner M, Mann M. **Deep proteomic evaluation of primary and cell line motoneuron disease models delineates major differences in neuronal characteristics**, *Mol Cell Proteomics. 2014, doi:10.1074/mcp.M113.037291*

4. Kulak NA, Pichler G, Paron I, Nagaraj N, Mann M. **Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells**, *Nature Methods 2014, doi:10.1038/nmeth.2834 15;37(1):46–56.*

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