

Protein extraction from Tissues and Cultured Cells using Bioruptor® Pico

Introduction

Protein extraction from tissues and cultured cells is the first step for many biochemical and analytical techniques (PAGE, Western blotting, mass spectrometry, etc.) or protein purification. Efficient disruption and homogenization of animal tissues and cultured cells are required to ensure high yields of proteins. Diagenode's Bioruptor® Pico uses state-of-the-art ultrasound technology to efficiently disrupt and homogenize tissues and cultured cells in just one step. The Bioruptor® Pico offers unique benefits for tissue disruption and homogenization:

- Fast and simple
- No contamination between samples
- Efficient
- Gentle processing
- Reproducible
- Temperature controlled
- Multiplexing capability

General remarks before starting

- Conditions for protein extraction (e.g. use of fresh or frozen tissue, composition of extraction buffer etc.) must be adjusted according to the nature of the proteins of interest and the assays to be run. SDS might be added to the extraction buffer to maximize the yield of soluble proteins. SDS extracts can be used for SDS electrophoresis and Western blotting. It is recommended to reduce the SDS concentration for 2D electrophoresis, enzyme-linked immunosorbent assay and mass spectrometry.
- For functional studies (e.g. the study of protein-protein interactions), avoid using ionic detergents and high concentrations of salt.

Extraction buffer: use RIPA buffer as a starting point for optimization:

50 mM Tris-HCl (pH 7.4)

150 mM NaCl

1% NP-40

0.25% Na-deoxycholate

Protease Inhibitor Mix

SDS 0.1 - 2% (optional)

It is always recommended to optimize the buffer composition depending on a specific research project

- Always use Protease Inhibitor Mix during extraction procedure to block the possible protein degradation.

- Use Diagenode's recommended tubes for sonication. Depending on the desired final volume, 1.5 ml Bioruptor® Microtubes with Caps (Cat. No.: C30010016) or 15 ml Bioruptor® Tubes (Cat. No.: C30010017) might be used. Always respect the recommended sonication volumes: 100 - 300 µl for 1.5 ml tubes and 1 - 2 ml for 15 ml tubes (strictly follow the Bioruptor® instructions as shown in the corresponding manual before starting any sonication experiments).
- Keep extracted proteins at -80°C.

Required materials and reagents

- Bioruptor® Pico (Cat. No. B0106001)
- 1.5 ml tube holder for Bioruptor® Pico (Cat. No. B01200040)
- 15 ml sonication accessories for Bioruptor® Pico (Cat. No. B01200016)
- 1.5 ml Bioruptor® Microtubes with Caps (Cat. No.: C30010016) or 15 ml Bioruptor® Tubes (Cat. No.: C30010017)
- Protein Extraction Beads (Cat No. C20000021) for tissue disruption (**not required for cell lysis**)
- Protease Inhibitor Mix (Cat. No. C12010012 or C12010011)
- Buffer for protein extraction from tissue or cell lysis (not supplied)
- Reagents for protein quantification (optional)

Protocol

I. Protein extraction from Tissues

- » *This protocol has been validated for up to 50 mg of tissue. Do not use more tissue per sample. For larger quantity cut the tissue and proceed to the disruption in separate tubes.*
- » *Minimize the time of tissue collection to prevent protein degradation.*
- » *Dissected tissues can be snap-frozen in liquid nitrogen and stored at -80°C until protein extraction*

1. Pre-cool Bioruptor® Pico to 4°C.
2. Fill the Bioruptor® tubes with Protein Extraction Beads.
 - » *The recommended quantity of the beads is 200 - 250 mg for 15 ml Bioruptor® Tubes, 40 - 50 mg for 1.5 ml Bioruptor® Microtubes.*
3. Add Protease Inhibitor Mix (200x) to the cold protein extraction buffer: 5 µl per 1 ml of extraction buffer. Scale accordingly.
4. Add the required volume of a cold extraction buffer to the Bioruptor® tubes filled with Protein Extraction Beads.
5. Add tissue pieces to the Bioruptor® tubes. Make sure that the final volume is in the recommended range: 100 - 300 µl for 1.5 ml Bioruptor® Microtubes and 1 - 2 ml for 15 ml Bioruptor® Tubes.

6. Vortex tubes briefly and proceed to sonication by using the Bioruptor® Pico with the following settings:

Sonication cycle: 30 sec ON/30 sec OFF

Total sonication time: 3 - 5 cycles

Temperature: 4°C

» *To guarantee homogeneity of sonication, the tube holder should be always completely filled with tubes.*

7. Stop the Bioruptor® Pico after each 2 cycles, vortex samples and check the sample visually for disruption.

» *Please note that the optimization might be required depending on the sample format (fresh or frozen tissue), tissue type and tissue amount. The shortest sonication time should be chosen to prevent protein damage. Incomplete disruption may occur with fibrous tissues (i.e. muscles).*

8. Transfer the supernatant to a new tube and centrifuge samples at 14,000 rpm for 15 min at 4°C to remove any remaining insoluble material.

» *The Protein Extraction Beads might be washed once with extraction buffer for maximum recovery of total protein but this will lead to the sample dilution.*

9. Transfer the supernatant containing soluble proteins to a new tube.

10. Take an aliquot for the quantification and the further analysis if needed. Store proteins extracts in small aliquots at -80°C.

» *Different protein quantification assays exist including: absorbance at 280 nm, Lowry Assay, Bradford Assay, Bicinchoninic Assay (BCA) etc.. Many commercial kits for protein quantification are also available. Please note that measuring the protein concentration in an SDS extract requires that the assay is compatible with the detergent and reducing agent in the solution.*

II. Protein extraction from Cultured Cells

» *This protocol has been validated using RIPA buffer but it may be necessary to optimize the buffer composition depending on a specific research project.*

» *We recommend using 100 µl of an appropriate lysis buffer per 1x10⁶ cells.*

» *For Western blotting, cells might be lysed directly in 1x Laemmli buffer. After sonication, centrifuge extract at 14,000 rpm for 15 min. Transfer the supernatant to a new tube and boil for 3 min. The supernatant can be used in Western blot. Note that protein quantification by common methods is not compatible with Laemmli buffer.*

1. Pre-cool Bioruptor® Pico to 4°C.
2. Add Protease Inhibitor Mix (200x) to the ice-cold cell lysis buffer: 5 µl per 1 ml of extraction buffer. Scale accordingly.

3. **For monolayer cells:**

Rinse the monolayer cells 3 times with cold PBS. For the final rinse, use a cell scraper and transfer the cell suspension to a Bioruptor® tube. Centrifuge cells at 1,500 rpm for 10 min at 4°C and aspirate as much supernatant as possible. Proceed to step 4.

For suspension cells:

Centrifuge suspension at 1,500 rpm for 10 min at 4°C and aspirate the supernatant. Resuspend the pellet in cold PBS, transfer to a Bioruptor® tube and centrifuge at 1,500 rpm for 10 min at 4°C. Aspirate the supernatant. Repeat 2 more times. Proceed to the step 4.

4. Add ice-cold cell lysis buffer and resuspend the pellet. Incubate on ice for 10 min.
 - » *The viscosity may appear at this step*
5. Vortex tubes briefly and proceed to sonication by using the Bioruptor® Pico with the following settings:

Sonication cycle: 30 sec ON/30 sec OFF

Total sonication time: 3 - 5 cycles

Temperature: 4°C

- » *To guarantee homogeneity of sonication, the tube holder should be always completely filled with tubes.*
6. Stop the Bioruptor® Pico after 2 cycles, briefly vortex samples and visually check the samples: Samples should be in solution (viscosity should be reduced)
 - » *Please note that the optimization might be required depending on sample format (cell density, cell type etc.). The shortest sonication time should be chosen to prevent protein damage.*
 7. Transfer the supernatant to a new tube and centrifuge samples at 14,000 rpm for 15 min at 4°C to remove any remaining insoluble material.
 8. Take an aliquot for the quantification and the further analysis if needed. Store protein extracts at -80°C.
 - » *Different protein concentration assays exist including: absorbance at 280 nm, Lowry Assay, Bradford Assay, Bicinchoninic Assay (BCA) etc. Many commercial kits for protein quantification are also available. Please note that measuring the protein concentration in an SDS extract requires that the assay is compatible with the detergent and reducing agent in the solution.*



Figure 1. Protein Extraction Beads are required for efficient tissue disruption using the Bioruptor®

Complete disruption is observed in the sample containing Diagenode's Protein Extraction Beads (left) after 5 cycles while non-disrupted tissue is still present in the sample without the Protein Extraction Beads (right).

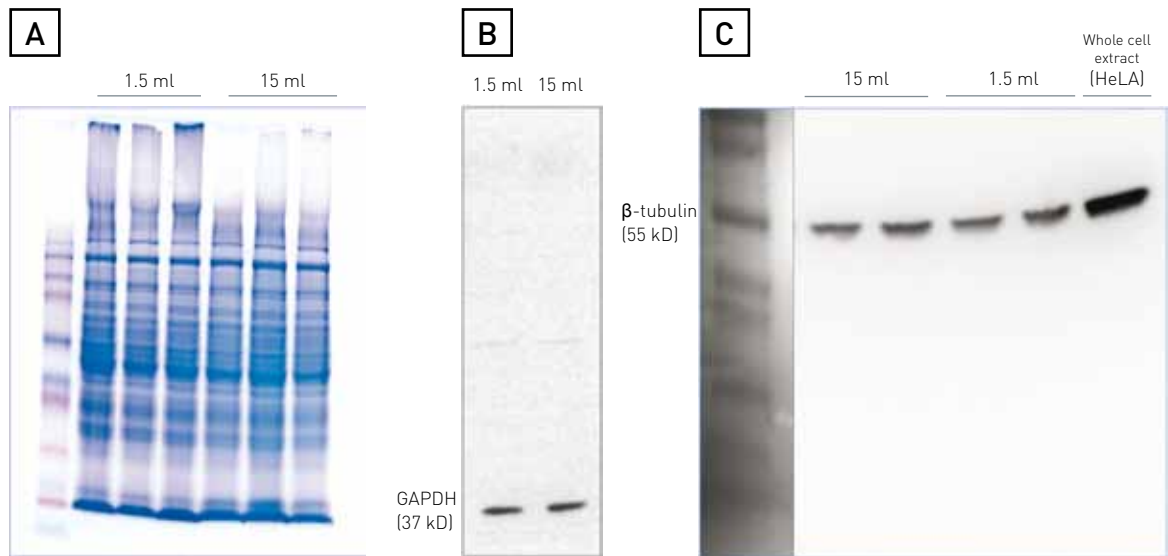


Figure 2. Total proteins effectively extracted from tissues using Bioruptor® Pico

30 mg of frozen mouse liver were disrupted in RIPA buffer in 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016) or 15 ml Bioruptor® Tubes (Cat. No. C30010016) supplemented with Protein extraction beads (Cat.No. C20000021) for 3 – 5 cycles with the Bioruptor® Pico (Cat.No. B01060001). Samples were centrifuged and analyzed by PAGE (Figure A) and Western blot using antibodies against GAPDH (Figure B) and beta tubulin (Figure C).