



Oct 21, 2023

Tissue Protein Extraction: Tissue Homogenization using Urea-based Buffer and Bead Mill Homogenizers

DOI

<https://dx.doi.org/10.17504/protocols.io.36wgg3op5lk5/v1>

J P Rose¹, M Watson¹, B Schilling¹, Joanna Bons¹

¹Buck Institute for Research on Aging



M A Watson

Buck Institute

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.36wgg3op5lk5/v1>

Protocol Citation: J P Rose, M Watson, B Schilling, Joanna Bons 2023. Tissue Protein Extraction: Tissue Homogenization using Urea-based Buffer and Bead Mill Homogenizers. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.36wgg3op5lk5/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 10, 2023

Last Modified: October 21, 2023

Protocol Integer ID: 89090

Keywords: Tissue protein extraction, Tissue homogenization, Proteomics, Mass Spectrometry, downstream proteomic profiling, bead mill homogenizers tissue homogenization, tissue homogenization, protein in preparation, protein, using urea, extraction, tissue

Abstract

Tissue homogenization to isolate protein in preparation for downstream proteomic profiling.



Materials

- Bead mill homogenizer
- Bicinchoninic acid (BCA) protein assay kit
- Delicate task wipers
- Metallic beads
- Tweezer
- 2-mL microcentrifuge tubes with the bead mill homogenizer
- 1.5-mL microcentrifuge tubes
- Urea
- 1 M triethylammonium bicarbonate (TEAB) solution, pH 8
- 30 mM nicotinamide solution in water
- 5 M sodium chloride solution in water
- HALT protease/phosphatase single-use inhibitor cocktail (100x) (Thermo Fisher Scientific, cat. number: 78440)
- 5 mM trichostatin A solution in water
- HPLC-grade water



- milliQ water
- HPLC-grade methanol

- 1 Chill the adaptor sets of the bead mill homogenizer at -20°C.
- 2 Freshly prepare the lysis buffer as described in **Table 1** and keep cold.

Reagent	Final Concentration	Amount
Urea	8 M	4.8 mg
TEAB, pH 8 (1 M)	200 mM	2 mL
Nicotinamide (30 mM)	3 mM	1 mL
Sodium chloride (5 M)	75 mM	150 µL
HALT protease/phosphatase single-use inhibitor cocktail (100x)	1 x	100 µL
Trichostatin A (5 mM)	1 µM	2 µL
HPLC-grade water	N/A	N/A
Total	N/A	10 mL

Table 1. Lysis buffer composition for a final volume of 10 mL.

- 3 On dry ice, add the tissue specimen to a 2-mL microcentrifuge tube compatible with the bead mill homogenizer, then place the metallic bead into the tube. Add 500 µL of cold lysis buffer to the tissue specimen (add more lysis buffer to cover the tissue specimen, if necessary).
- 4 Place the tubes in the prechilled homogenizer adaptor sets, ensuring that the tubes are balanced between the two adaptors.
- 5 Homogenize the samples for 2 cycles at 24 Hz for 2 min each. If the tissue specimen is not fully homogenized, spin the samples briefly, transfer the homogenized lysate into a clean 1.5-mL microcentrifuge tube, add additional lysis buffer, and repeat the homogenization step. Two cycles of homogenization are typically enough to break up the tissue.



- 6 Remove the bead with a tweezer. In between each sample, rinse the tweezer with milliQ water, then HPLC-grade methanol, and dry thoroughly with a delicate task wiper.
- 7 Transfer the homogenized lysate into a clean 1.5-mL microcentrifuge tube or combine the homogenized lysates if needed, carefully avoiding bubbles and the separated fat layer at the top of the supernatant.
- 8 Centrifuge the homogenized lysate at 15,700 x g for 15 min at 4°C to clear the lysate.
- 9 Transfer the supernatant (clear lysate containing the soluble proteins) to a clean 1.5-mL microcentrifuge tube, carefully avoiding any liquid fat layer on top and debris at the bottom of the tube.
- 10 Perform a bicinchoninic acid (BCA) protein assay to determine the protein concentration of the clear lysate with a proper dilution (typically 1:20 - 1:200 depending on the tissue type). According to the BCA results, transfer an aliquot of the sample into a clean 2-mL microcentrifuge tube for subsequent proteolytic digestion.