



OCT 20, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.36wgq3op5lk5/v1

Protocol Citation: J Bons, J P Rose, M A Watson, B Schilling 2023. Tissue Protein Extraction: Tissue Homogenization using Urea-based Buffer and Bead Mill Homogenizers. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.36wgq3op5lk5/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

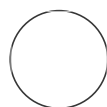
Created: Oct 10, 2023

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

M A

J Bons¹, J P Rose¹, Watson¹, B Schilling¹

¹Buck Institute for Research on Aging



M A Watson

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 triethylamonium bicarbonate (TEAB) solution, pH 8

Last Modified: Oct 20, 2023

PROTOCOL integer ID:
89090

Keywords: Tissue protein
extraction, Tissue
homogenization, Proteomics,
Mass Spectrometry

- 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

Reagent	Final Concentration	Amount
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 μ L 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.