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We use this protocol and it's working

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

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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

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- 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 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.