



The following instructions are applicable for **diluted lyophilized mouse tissue in**

Urea buffer

SDS buffer

Operating instructions

1. Add 450 μ l distilled H₂O to the lyophilized tissue.
2. Resuspend the tissue until no more powder is visible.
The resulting solution contains: 100 μ g protein
6 M Urea
2 M Thiourea
10 mM HEPES pH 7.4
3. Spin down the foam at 10 000 g for 10 min at room temperature.
4. Gently resuspend the pellet. Avoid foaming.
Comment: The tissue contains insoluble components (e.g. cell debris containing membrane proteins).

For the further procedure we recommend the methods as described in:

- Geiger, T. et al.¹
- Hölper, S. et al.²

Our recommendation for the preparation of the spike-in:

1. Add DTT to a final concentration of 0.1 M.
2. Incubate at 95 °C for 5–10 min.
3. Sonicate for 20 s (duty cycle 20%).
4. Spin down for 5 min at 16 000 g at room temperature.
5. Estimate protein concentration of the supernatant using established methods that tolerate high urea and high DTT concentrations.
6. Mix the tissues with a protein amount ratio of 1:1

¹ Geiger, T., Wisniewski, J., Cox, J. et al. Use of stable isotope labeling by amino acids in cell culture as a spike-in standard in quantitative proteomics. *Nat Protoc* 6, 147–157 (2011) doi:10.1038/nprot.2010.192.

² Hölper S., Ruhs A., Krüger M. (2014) Stable Isotope Labeling for Proteomic Analysis of Tissues in Mouse. In: Warscheid B. (eds) Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC). *Methods in Molecular Biology (Methods and Protocols)*, vol 1188. Humana Press, New York, NY.

This product is for laboratory use only. The safety and efficacy of this product in diagnostic or other clinical uses is not established.