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

Urea buffer SDS buffer

Operating instructions

1. *Add 150 µl distilled H_2O to the lyophilized tissue.*
2. *Resuspend the tissue until no more powder is visible.*
   The resulting solution contains: 100 µg 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.