



QIAGEN Supplementary Protocol:

Purification of protein from animal tissues using the Qproteome Mammalian Protein Prep Kit and the TissueRuptor™

Important points before starting

- Ensure that you are familiar with operating the TissueRuptor. Refer to the *TissueRuptor User Manual* for operating instructions.
- Disruption in lysis buffer may lead to foaming. Use a suitably sized vessel for disruption. If foaming occurs, let the sample stand on ice or at 2–8°C for a few minutes or centrifuge the sample briefly before continuing with the purification protocol. Some foams are so persistent that it is not possible to spin them down. In such cases antifoam Reagent DX (cat. no. 19088) can be used. Disruption for more than 15 seconds may generate heat, depending on the disruption vessel used.

Procedure

1. **Place a 40 mg tissue sample into a suitably sized vessel and add 1 ml of Mammalian Cell Lysis Buffer including Benzonase® Nuclease and Protease Inhibitor Solution.**

Note: For efficient disruption, the size of the tissue sample must not be greater than half the diameter of the TissueRuptor probe. Cut the sample into smaller pieces, if necessary.

2. **Place the tip of the disposable probe into the tube containing the sample and lysis buffer. To avoid damage to the TissueRuptor and probe during sample disruption, make sure that the tip of the probe is submerged in the buffer.**

Important: Use a minimum of 180 µl lysis buffer in a suitably sized vessel.

3. **Disrupt for 30 s at medium speed. Move the tip of the probe within the sample vessel during processing to increase the efficiency of disruption and homogenization.**
4. **Centrifuge the suspension for 10 min at 14,000 x g in a precooled microcentrifuge at 4°C.**

5. **Transfer the supernatant into a new microcentrifuge tube.**

The sample is ready for downstream applications such as fractionation using Qproteome kits, 1D-PAGE, immunoprecipitation, or activity assays.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.aspx.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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