QIAGEN Supplementary Protocol:

Acetone precipitation of protein from Buffer RLT or Buffer RLT Plus lysates

This protocol is designed for acetone precipitation of protein from cell lysates prepared using Buffer RLT (supplied with RNeasy® Kits) or Buffer RLT Plus (supplied with the RNeasy Plus Mini Kit and the AllPrep DNA/RNA Mini Kit). The precipitated, denatured protein is suitable for applications such as SDS-PAGE, western blotting, and 2D gel electrophoresis.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the handbook supplied with the RNeasy Kit or the AllPrep DNA/RNA Mini Kit before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Equipment and reagents to be supplied by user

- Ice
- Benchtop centrifuge
- Acetone
- Optional: Ethanol
- Buffer for downstream application (e.g., loading buffer for SDS-PAGE gel)

Important point before starting

- **DO NOT use trichloroacetic acid (TCA) to precipitate protein from Buffer RLT or Buffer RLT Plus lysates.** These buffers contain guanidine thiocyanate, which can form highly reactive compounds when combined with acidic solutions.

Procedure

1. **Prepare cell lysate and centrifuge it through an RNeasy spin column, as described in the handbook supplied with the RNeasy Kit or the AllPrep DNA/RNA Mini Kit.**
2. **Add 4 volumes of ice-cold acetone to the flow-through from the RNeasy spin column.**
3. **Incubate for 30 min on ice or at –20°C.**
4. **Centrifuge for 10 min at maximum speed in a benchtop centrifuge. Discard the supernatant and air-dry the pellet.***

* Supernatant contains guanidine thiocyanate and is therefore not compatible with bleach. See the handbook supplied with the RNeasy Kit or the AllPrep DNA/RNA Mini Kit for safety information.

5. **Optional: Wash the pellet with 100 µl ice-cold ethanol and air-dry.**
   Do not overdry the pellet as this may make resuspension more difficult.

6. **Resuspend the pellet in the buffer for your downstream application.**
   Sodium dodecyl sulfate (SDS) causes guanidine salts to precipitate. In case the pellet contains traces of guanidine thiocyanate, load the sample onto an SDS-PAGE gel immediately after heating for 7 minutes at 95°C.

QIAGEN kit handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected kit handbooks can be downloaded from www.qiagen.com/literature .
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp .