Quick-Start Protocol March 2016

Qproteome® Cell Compartment Kit

The Qproteome Cell Compartment Kit (cat. no. 37502) components should be stored at the following temperatures: Benzonase® Nuclease and Extraction Buffers CE1, CE2 and CE3 at –30 to –15°C, Protease Inhibitor Solution (100x) at 2–8°C and Extraction Buffer CE4 at room temperature (15–25°C).

Further information

- *Qproteome Cell Compartment Handbook*: **www.qiagen.com/HB-0858**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is suitable for processing of 5 x 10^6 cells.
- The extraction buffers contain components that may interfere with protein quantification assays. A precipitation step to remove interfering substances is required to accurately determine protein concentrations.

1. Thaw Protease Inhibitor Solution (100x) and Extraction Buffers CE1, CE2 and CE3. After thawing, mix well by vortexing and place on ice. For each fractionation procedure, prepare the volume of buffer supplemented with Protease Inhibitor Solution (100x) given in the table below.
2. Transfer a cell suspension containing 5 x 10^6 cells into a 15 ml conical tube and centrifuge at 500 x *g* for 10 min at 4°C. Carefully discard supernatant.
3. Resuspend the cell pellet in 2 ml ice-cold PBS by pipetting and transfer the cell suspension into a microcentrifuge tube. Pellet cells by centrifuging at 500 x *g* for 10 min at 4°C. Carefully discard supernatant.
4. Repeat step 3.

5. Resuspend the cell pellet in 1 ml ice-cold Extraction Lysis Buffer by pipetting. Incubate for 10 min at 4°C on an end-over-end shaker.
6. Centrifuge the lysate at 1000 x *g* for 10 min at 4°C.
7. Carefully transfer the supernatant (fraction 1) into a fresh microcentrifuge tube. Store on ice. This fraction primarily contains cytosolic proteins.
8. Resuspend the pellet in 1 ml ice-cold Extraction Buffer CE2 by pipetting. Incubate for 30 min at 4°C on an end-over-end shaker.
9. Centrifuge the suspension at 6000 x *g* for 10 min at 4°C.
10. Carefully transfer the supernatant (fraction 2) into a fresh microcentrifuge tube. Store on ice. This fraction primarily contains membrane proteins.
11. Add 7 µl Benzonase® Nuclease and 13 µl distilled water to the pellet. Resuspend the pellet by gently flicking the bottom of the tube. Incubate for 15 min at room temperature (15–25°C).
12. Pipet 500 µl ice-cold Extraction Buffer CE3 into the tube and mix by pipetting. Incubate for 10 min at 4°C on an end-over-end shaker.
13. Pellet insoluble material by centrifuging at 6800 x *g* for 10 min at 4°C.
14. Transfer the supernatant (fraction 3) into a fresh sample tube. Store on ice. This fraction primarily contains nuclear proteins.
15. Resuspend the pellet from step 13 in 500 µl Extraction Buffer CE4. Label the suspension fraction 4. This fraction primarily contains cytoskeletal proteins.

Table 1. Volume of buffer required for each fractionation procedure

	Lysis Buffer	Buffer CE2	Buffer CE3	Buffer CE4
Required volume	1 ml	1 ml	0.5 ml	0.5 ml
Protease Inhibitor Solution (100x)	10 µl	10 µl	5 µl	–

Scan QR code for handbook.