

Protein Expression Sample Preparation

Note: For safety and biohazard guidelines, refer to the “Safety” section in the *Protein Expression Sample Preparation Protocol* (PN 4405785). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1 Prepare the cell lysis solution



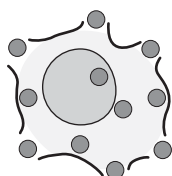
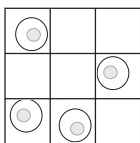
- a. Reconstitute the **Calbiochem® Protease Inhibitor Cocktail Set I** by adding 1 mL H₂O, briefly vortex the solution, then put the vial on ice.
- b. Pipette into a tube, in the order shown:

Cell lysis solution	Volume per sample (µL)
Cell Lysis Reagent, 2X	500
Calbiochem® Phosphatase Inhibitor Cocktail Set II, 100X	5
Calbiochem® Protease Inhibitor Cocktail Set I, 100X	5
Total Volume	510

- c. Touch vortex, then briefly centrifuge the tube.
- d. Put the cell lysis solution on ice.

IMPORTANT! Perform cell lysis immediately. Discard unused solution after 2 hours.

2 Count and lyse the cells



For each sample:

- a. Collect the sample cells using your laboratory protocol.
- b. Count the cells, using hemocytometry.
- c. Centrifuge the cells at 500 × g for 4 min, then rinse two times with PBS.
- d. Centrifuge the cells again, carefully remove all of the supernatant, then resuspend the cell pellet in sufficient ice-cold Cell Resuspension Buffer to achieve a concentration of 1000 cells/µL. If the total volume is >500 µL, transfer 500 µL to a fresh tube.
- e. Add a volume of cell lysis solution equal to the volume in the tube to achieve a concentration of 500 cells/µL.
- f. Pipette the solution up and down several times, avoiding excess detergent foaming.

3 (Optional) Store the lysates

Applied Biosystems recommends that you perform the TaqMan® Protein Expression Assay soon after you prepare the cell lysates.

If you do not proceed immediately to the TaqMan® Protein Expression Assay, divide the cell lysates into volumes of about 50 µL, then store the lysates at ≤ -50 °C (e.g., -70 °C).

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06/2009

