Cat # SL100319 Store at 4 °C

CompLysis™ Protein Extraction Reagent for Mammalian Cells

☐ **Small** **125 ml**

☐ **Large** **500 ml**

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This product is for laboratory research ONLY and not for diagnostic use

Description:

The CompLysis™ Mammalian Cell Extraction Reagent provides optimized cell extraction buffer for convenient extraction of mammalian proteins from cultured cells and tissue samples, under nondenaturing conditions. Cell lysate prepared using the reagent can be used in a variety of applications, such as enzyme activity assays (e.g., caspase activity assays), Western blot analysis, and others. The entire procedure takes less than 10 minutes.

General Consideration and Reagent Preparation:

• After opening the cap, store CompLysis™ Protein Extraction Reagent at 4 0C.
• During cell lysis, series of protease inhibitors are necessary to add into the buffer for complete inhibition of protease activity. We provide TotalX™ Protease Inhibitor Cocktail (catalog # SL100315 and SL100316) which is supplied as lyophilized form. To reconstitute, add 1 ml of this reagent to the vial, pipette several times to dissolve all powder. This makes 100X concentrated Protease Inhibitor Cocktail.
• Before use, dilute TotalX™ Protease Inhibitor Cocktail (catalog # SL100315 and SL100316) with CompLysis™ Protein Extraction Reagent.
• Be sure to keep the Extraction Buffer Mix on ice at all times during the experiment.

Mammalian Protein Extraction Protocol:

Protocol I: Lysis of Adherent Mammalian Cells

• Carefully remove (decant) culture medium from the adherent cells.
• Optional wash: If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash the cells once with PBS.
• Add an appropriate volume of CompLysis™ Protein Extraction Reagent with protease inhibitors added to the cells (see Table 1) and let cells sit on ice for 5 minutes. Scrap the cells with a cell scraper.
• Incubate on ice for 5 minutes, then vertex for 5 seconds.
• Collect the lysate and transfer to a microcentrifuge tube. Centrifuge samples at
27,000 g for 5~10 minutes to pellet the cell debris.
• Transfer supernatant to a clean tube for further analysis.

Table 1: Suggested Volume of CompLysis™ Protein Extraction Reagent to use for Different Sizes of Standard Culture Plates

Plate Size	Volume (µl)
100 mm	700 µl~1,000 µl
60 mm	400 µl ~600 µl
6-well plate	300 µl ~500 µl per well
24-well plate	150 µl ~300 µl per well
96-well plate	80 µl ~150 µl per well

Protocol II: Lysis of a Mammalian Cell Suspension

• Pellet the suspension of cells by centrifugation at 2,500g for 10 minutes. Discard the supernatant.
• Optional wash: If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash the cells once by resuspending the cell pellet in a desired wash buffer (e.g., PBS). Pellet the cells by centrifugation at 2,500g for 10 minutes.
• Add CompLysis™ Protein Extraction Reagent to the cell pellet. At least 10 ml of CompLysis™ Protein Extraction Reagent is recommended for each 1 gram of wet cell pellet.

Note: If a large amount of cells is used, first add 1/10 the final recommended volume of CompLysis™ Protein Extraction Reagent to the cell pellet. Pipette the mixture up and down to resuspend the pellet. Add the rest of the CompLysis™ Protein Extraction Reagent to the cell suspension.
• Shake gently for 10 minutes, remove cell debris by centrifugation at 27,000g for 15 minutes.
• Transfer supernatant to a clean tube for further analysis.

Storage: Upon arrival store this product at 4 °C. Product shipped at ambient temperature.