CREATIVE BIOMART®
Assay Kit

Total Protein Extraction Kit

Product Information

Common Name

Protein

Cat.No. Kit-2529

Product Overview

The Total Protein extracon Kit provides an opmized cell lysis buffer, protease inhibitor cocktail, and Phosphatase inhibitor for convenient extracon of mammalian proteins from cultured cells and ssue samples, under non□denaturing condions. Total protein isolated by this kit maintain biological acvity and can be used for many downstream applicaons including SDS□PAGE, Western Blong, IP, Pull Down, Gel Mobility Shi, protein assays and so on. The kit is sufficient to extract proteins from 50x10^7 mammalian cells or 50x200mg of ssues sample.

Storage

Transportaon at room temperature. Upon receipt, store Lysis buffer at 4°C.
Keep Protease Inhibitor Phosphatase Inhibitor, and PMSF at □20°C.

Size

50 preps

Kit Components

Lysis Buffer 50ml
Protease Inhibitor 50 ul
Phosphatase Inhibitor 250 ul
PMSF 500 ul

Technical Notes

All reagents and instruments must be pre□chilled, so that the extracted protein can remain acve and intact.
If low protein concentraon in total Protein Fracon is observed, cell lysis may not be efficient. Please increase the number of strokes of the homogenizer. In the meanme, add 1ul Protease inhibitor and

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5ul Phosphatase inhibitor, 10ul PMSF into 1ml Lysis Buffer before use.

Assay Protocol

1. Wash the cells with ice□cold PBS three mes from cell suspension culture. Add ice□cold PBS and scrape the cells off the dish using a cell scraper. Transfer the cells to a pre□chilled centrifuge tube and spin at 3000rpm for 5 minutes at 4°C. Remove supernatant and wash cell pellet by gently re□suspending with ice□cold PBS. Spin at 3000rpm for 5 minutes at 4°C and remove supernatant. Each extracon requires about 1x10^7 cells.
Note: For ssue samples, each extracon requires 100□200mg sample in weight. Remove as much as fat and nerve ssue as possible, then cut ssue sample into small pieces, and then wash them with pre□chilled PBS three mes.
2. Add 1ml ice cold Lysis Buffer (before use, add 1ul Protease inhibitor and 5ul Phosphatase inhibitor, 10ul PMSF into 1ml Lysis Buffer), vortex. Homogenize mixture with glass homogenizer for 30□50 strokes or sonicate for 30 seconds, 1 minute interval. Repeat operaon three mes. Check the efficiency of cell lysis and ensure more than 90 percent cells have been broken.
3. Transfer the above homogenized soluon into a new pre□chilled 1.5ml centrifuge tube , place the tube on ice for ten minutes. Occasionally vortex 3□4 mes, then centrifuge at 14000rpm for 5 minutes at 4°C, discard precipitates and keep supernatant as total Protein Fracon at □80°C for IP assays, protein assays, reporter assays and other immunoassay procedures.

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