

## Product information

# Total Protein Extraction Kit

|                   |   |
|-------------------|---|
| <b>Catalog #:</b> | BSP003  |
| <b>Size:</b>      | 50 preps  |
| <b>Storage:</b>   | -20°C (Protease Inhibitor + Phosphatase Inhibitor + PMSF), -4°C (Lysis buffer ) |

### Description:

The Total Protein extraction Kit provides an optimized cell lysis buffer, protease inhibitor cocktail, and Phosphatase inhibitor for convenient extraction of mammalian proteins from cultured cells and tissue samples, under non-denaturing conditions. Total protein isolated by this kit maintain biological activity and can be used for many downstream applications including SDS-PAGE, Western Blotting, IP, Pull Down, Gel Mobility Shift, protein assays and so on. The kit is sufficient to extract proteins from  $50 \times 10^7$  mammalian cells or  $50 \times 200$ mg of tissues sample.

| Kit Components        | QTY    |
|-----------------------|--------|
| Lysis Buffer          | 50 ml  |
| Protease Inhibitor    | 50 ul  |
| Phosphatase Inhibitor | 250 ul |
| PMSF                  | 500 ul |
| Protocol              | 1 pc   |

### Storage and Transportation:

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

### Procedure:

1. Wash the cells with ice-cold PBS three times 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 extraction requires about  $1 \times 10^7$  cells.

**Note:** For tissue samples, each extraction requires 100-200mg sample in weight. Remove as much as fat and nerve tissue as possible, then cut tissue sample into small pieces, and then wash them with pre-chilled PBS three times.

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 operation three times. Check the efficiency of cell lysis and ensure more than 90 percent cells have been broken.
3. Transfer the above homogenized solution into a new pre-chilled 1.5ml centrifuge tube , place the tube on ice for ten minutes. Occasionally vortex 3-4 times, then centrifuge at 14000rpm for 5 minutes at 4°C, discard precipitates and keep supernatant as total Protein Fraction at -80°C for IP assays, protein assays, reporter assays and other immunoassay procedures.

### Important Notes:

- All reagents and instruments must be pre-chilled, so that the extracted protein can remain active and intact.
- If low protein concentration in total Protein Fraction is observed, cell lysis may not be efficient. Please increase the number of strokes of the homogenizer. In the meantime, add 1ul Protease inhibitor and 5ul Phosphatase inhibitor, 10ul PMSF into 1ml Lysis Buffer before use.