Total Protein Extraction Kit(strong)

Cat: BC3710
Size: 50T /100T
Validity: At least 1 year.

Kit Components:

Kit Components	50T	100T	Storage
Lysate(strong)	50mL	100mL	2-8℃
Phosphatase inhibitors(100×)	0.5mL	1mL	-20℃
Protease inhibitor(100×)	0.5mL	1mL	
PMSF(100×)	0.5mL	1mL	

Introduction:

This kit is used to extract total protein from mammalian tissues and cells, the lysate in the kit contains protease inhibitors and phosphatase inhibitors, the effect is relatively strong, can quickly obtain total protein, can be used for western blot experiments and other basic research experiments, because contains the above enzyme inhibitors, can not be used to study protein kinase and phosphokinase research. This product is only used for scientific research.

Protocols(only for reference):

*** Add 10uL each of phosphatase inhibitors, protease inhibitors and PMSF to 1mL of cold lysate; Mix well, place on ice and set aside;**

1. Extraction of total protein from tissues

1) Weigh 0.1g fresh tissue, place it at the inlet buffer of the glass homogenizer, cut the tissue block as much as possible with ophthalmic scissors, then add 0.5-1mL newly configured lysate, grind until there is no obvious tissue block, this process pay attention to the operation on the ice;

2) Transfer the tissue homogenate into 1.5mL EP tube and centrifuge at 4℃, 12000g for 30min;

3) Absorb the supernatant into the new tube;

4) And carry out protein quantification or denaturation for protein experiments.

(The extracted protein is recommended to be stored at -80℃, ready to use, to avoid repeated freezing and thawing, to avoid long-term storage.)

2. Extraction of total cell protein

1) The amount of lysate: 10^7 cells need lysate 1mL;

2) Adherent cells: discard the medium, wash twice with cold PBS, discard PBS, and then add the calculated cell lysate; Scrape the cells off with a cell scraper on the ice, transfer the scraped cell lysate into the EP tube, reverse cleavage for 20-30min.

3) Suspension cells: Centrifuge cells at 4℃, 400g, wash the cells twice with cold PBS, add the lysate again according to the number of cells, swirl for 10s, place on ice for cleavage for 10min, repeat 3-4 times;

4) After the lysis, the cell lysate was centrifuged at 4℃, 12000g for 30min;

5) Transfer the supernatant into a new EP tube; Protein quantification or denaturation is performed for protein experiment.

(The extracted protein is recommended to be stored at -80℃, ready to use, to avoid repeated freezing and thawing, to avoid long-term storage.)

Notes:

1. During the experiment, all reagents need to be pre-cooled or melted to ensure the low temperature environment during the operation.
2. PSMF (toxic) can be added as soon as possible because PMSF degrades rapidly in aqueous solution.