

Phosphorylated Protein Extraction Kit

Cat: BC3730

Size: 50T/100T

Validity: At least 1 year.

Kit Components:

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

Introduction:

This kit is used to extract cytoplasmic protein from mammalian tissues and cells. The lysate in the kit contains protease inhibitors and phosphatase inhibitors, which have a strong effect and can obtain total protein. It can be used for basic research experiments such as western blot experiments. This product is only used for scientific research.

Protocols(only for reference):

Add 10μL each of phosphatase inhibitor, protease inhibitor and PMSF to 1mL cold lysate; Mix well and place on ice for later use;

1. Extraction of tissue total protein

- 1) Weigh 0.1g fresh tissue and place it at the inlet buffer of the glass homogenizer. Cut the tissue blocks as much as possible with ophthalmic scissors, then add 0.5-1mL newly configured lysate and grind until there are no obvious tissue blocks. This process should pay attention to the operation on the ice;
- 2) Transfer the tissue homogenate into 1.5mL EP tube and centrifuge at 4°C at 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°C, 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°C, 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, centrifuge the cell lysate at 4°C, 12000g for 30min;
- 5) Transfer the supernatant into a new EP tube; Protein quantification or denaturation was performed for protein experiment.

(The extracted protein is recommended to be stored at -80 °C , 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.