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Phosphorylated Protein Extraction Kit

Cat No: BC3730

Size: 50T/100T

Validity: At least one year.

Kit contents:

	50T	100T	Storage
Lysis buffer	50 ml	100ml	2-8℃
Phosphatase inhibitor(100×)	0.5ml	1ml	-20℃
Protease inhibitor(100×)	0.5ml	1ml	-20℃
PMSF(100×)	0.5ml	1ml	-20℃

Description:

Phosphorylated Protein Extraction Kit is used to extract cytoplasmic proteins from mammalian tissues and cells. Lysis buffer contains protease inhibitor and phosphatase inhibitor, which have strong effects. Total protein can be obtained, which can be used for Western Blot and other basic researches. Because it contains above-mentioned inhibitors, it cannot be used to study of protein kinase and phosphokinase. This product is for scientific research only.

Operation procedure

Add 10μl phosphatase inhibitor, protease inhibitors and PMSF to 1ml cold lysis buffer, mix thoroughly, place the mixture on ice;

1. Extraction of tissues total protein

1) Weigh 0.1g fresh tissue, place at the upper entrance area of the glass homogenizer, and use ophthalmic scissors to cut the tissue block as much as possible. Add 0.5-1ml newly prepared lysis buffer, grind it until there is no obvious tissue block, this step needs to be operated on ice;
2) Transfer tissue homogenate to 1.5ml EP tube, and centrifuge at 12000g for 30min at 4℃;
3) Transfer supernatant to a new tube;
4) Proceed protein quantification or denaturation for further protein experiments. (The extracted protein is recommended to be stored at-80℃, avoid repeated freezing and thawing or long time storage.)

2. Extraction of cells total protein

1) The volume of lysis buffer:10^7 cells need 1ml lysis buffer.
2) Adherent cells: discard culture medium, wash cells twice with cold PBS solution, discard PBS solution, then add proper lysis buffer; scrape cells with cell scraper on the ice, and transfer lysis solution to a new EP tube and inverted tubes repeatedly for 20-30 min.

3) Suspension cells: collect cells by centrifuge at 400g at 4℃, wash cells twice with cold PBS solution, then add proper lysis buffer, and vortex cells for 10s, placed on ice for 10min, repeat 3-4 times.
4) After completed lysis, centrifuge lysis solution at 12000g for 30min at 4℃.
5) Transfer supernatant to a new EP tube and proceed protein quantification or denaturation for further protein experiments.
   (The extracted protein is recommended to be stored at -80℃, avoid repeated freezing and thawing or long time storage.)

Note

1. In the experimental process, all reagents need to be pre-cooled to ensure a low temperature environment during operation.
2. PSMF(toxic) is only added in lysis buffer before use, because PMSF will degrade rapidly in aqueous solution.