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Endochylema Protein Extraction Kit

Cat No: BC3740

Size: 50T/100T

Validity: at least one year.

Kit contents

	50T	100T	Storage
Lysis buffer(highly active)	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:

The Endochylema Protein Extraction Kit is designed to extract endochylema protein from mammalian tissues and cells. The lysis solution contains protease inhibitor and phosphatase inhibitor, which could be used to obtain the total protein mildly. As enzyme inhibitor mentioned above, this kit could be used for Western blotting experiments and other basic researches, not for researches about protein kinase and phosphokinase.

Protocol

1. Extraction of tissue endochylema protein

1) Add 10μl phosphatase inhibitor, protease Inhibitors, PMSF to 1ml cold lysis buffer, place the mixture on the ice;
2) Cut 0.1g fresh tissue in small and add 0.5-1ml newly prepared Lysis buffer, grind it until there is no obvious tissue block. This process needs to be operated on ice all the time;
3) Transfer the tissue homogenate to 1.5ml EP tube, and centrifuge at 12000g for 30min at 4℃;
4) Transfer the supernatant to a new tube;
5) Quantitative or denatured proteins were used for further protein experiments
(The extracted protein is recommended to be stored at-80 ℃, avoid repeated freezing and thawing, and to avoid long time storage.)

2. Extraction of cell endochylema protein

1) The amount of the lysis solution: 10^7 cells need 1ml lysis buffer ;
2) Adherent cells: discard the culture medium, wash cells twice with cold PBS , discard the PBS, then add proper lysis buffer; scrape cells with cell scraper on the ice , and the scraped cell lysate was transferred to an EP tube and inverted for 20-30 min.
3) Suspension cells: collect cells by centrifugation at 4℃, 400g, wash cells with cold PBS twice, then add proper lysis buffer; and vortex cells for 10 s, placed on ice for 10 min, repeated 3-4 times;
4) After the lysis is completed, centrifugate the cell lysate at 12000 g for 4 min at 30 ℃;

5) Transfer the supernatant to a new tube and quantitative or denature proteins were used for further protein experiments. (The extracted protein is suggested to be kept at -80℃ with small package. Avoid freeze/thaw cycles and long time storage)

Note

1. All reagents should be pre-cooled or melted, keep in low temperature during operation.
2. PSMF (toxic) is added to lysis buffer before use, because PMSF will degrades rapidly in aqueous solution.