Total Protein Extraction Assay Kit

Catalog No: E-BC-E002 **Specification:** 50 Assays/100 Assays

Note: ① It is recommended to take samples before preparing the lysis working solution.

② The whole process of extraction should be in an ice bath or low temperature.

General information

Intended use This kit can be used to extract total protein from animal tissues and cells, and the obtained protein can be used for subsequent studies such as Western Blot and co-immunoprecipitation.

Detection principle Cell and tissue samples are treated with lysates containing protease inhibitors and phosphatase inhibitors to prevent the enzymes in the sample from being released to hydrolyze or dephpsphprylate protein due to the disruption of the membrane system.

Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Lysis Buffer	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Phosphatase Inhibitor	0.6 mL × 1 vial	1.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Protease Inhibitor	0.6 mL × 1 vial	1.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Phenylmethylsulfonyl Fluoride	0.6 mL × 1 vial	1.2 mL × 1 vial	-20°C, 12 months, shading light

Note: The reagents must be stored strictly according to the preservation conditions in the above table.

The reagents in different kits cannot be mixed with each other.

Materials prepared by users

Instruments:

High-speed freezing centrifuge, 5 mL Glass homogenizer

Reagents:

Double distilled water, PBS(0.01 M，pH 7.4)

Reagent preparation

① Place all reagents in ice water for pre-cooling for at least 15 min until it has returned to the solution state before use.

② The preparation of lysis working solution:

Before testing, please prepare sufficient lysis working solution. For example, prepare 1020 μL of lysis working solution (mix well 1000 μL of lysis buffer, 10 μL of phosphatase inhibitor and 10 μL of protease inhibitor). Keep it \on ice during use protected from light and the prepared solution should be used within 20 min.

Operation table

1. Total Protein Extraction of Tissue

① Take 0.1 g of fresh tissue, wash with PBS（0.01 M，pH 7.4） at 2-8℃ to remove blood. Blot the water with absorbent paper.

② Cut the tissue into pieces with scissors and place them into a pre-cooled 5 mL glass homogenizer.

③ Add 1 mL of pre-cooled lysis working solution and 10 μL of precooled phenylmethylsulfonyl fluoride.

④ Grind the tissue up and down in the ice bath for about 30 times.

⑤ Transfere tissue homogenate to a 2 mL pre-cooled EP tube, place in the ice bath for 15 min.

⑥ Centrifuge at 12000×g at 4°C for 15 min. The supernatant was the total protein extract. Place it on ice for detection.

⑦ The prepared total protein solution should be stored at -70°C with avoiding of repeated freeze-thaw.

2. Total Protein Extraction of Cell

a、Cell collection

Suspension cell: Transferre cell suspension to pre-cooled EP tubes, centrifuge at 4°C at 1000×g for 10 min to remove supernatant, wash with PBS（0.01 M，pH 7.4） at 2-8°C once, centrifuge at 4°C at 1000×g for 10 min to remove supernatant, leaving precipitation for use.

Adherent cell: Discard the culture solution and wash the cells twice with PBS (0.01 M, pH 7.4) at 2-8°C. Scrape down cells with cell scraping, or treat with EDTA solution, blown the cells off with a pipettor and transferre the cell suspension to a pre-cooled EP tube. Centrifuge at 4°C at 1000×g for 10 min to remove supernatant, wash with PBS（0.01 M，pH 7.4）at 2-8°C once, centrifuge at 4°C at 1000×g for 10 min to remove supernatant, leaving precipitation for use.

b、Cell extraction

① Take 5×10^6 cells and add 0.5 mL of pre-cooled lysis working solution and 10 μL of precooled

phenylmethylsulfonyl fluoride.

② Place on ice box for 15 min, vortex and mix every 5 min for 10 s each time.

③ Centrifuge at 12000×g at 4°C for 15 min. The supernatant was the total protein extract, which was placed on ice for detection.

④ The prepared total protein supernatant should be stored at -70°C to avoid repeated freeze-thaw.