TECHNICAL MANUAL

Total Protein Extraction Kit

- **SKU CODE:** MAES0255
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. General information

Note:

① It is recommended to collect samples before preparing the lysis working solution.

② The entire extraction process should be performed in an ice bath or at low temperature.

2. Intended use

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

3. Detection principle

Cell and tissue samples are treated with lysis buffer containing protease inhibitors and phosphatase inhibitors to prevent enzymes in the sample from being released to hydrolyze or dephosphorylate proteins due to disruption of the membrane system.

4. 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, protect from light
Reagent 3	Protease Inhibitor	0.6 mL × 1 vial	1.2 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 4	Phenylmethylsulfonyl Fluoride	0.6 mL × 1 vial	1.2 mL × 1 vial	-20°C, 12 months, protect from light

5. Materials prepared by users

Instruments:

High-speed refrigerated centrifuge, 5 mL glass homogenizer

Reagents:

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

Note: The reagents must be stored strictly according to the preservation conditions in the above table. Reagents from different kits cannot be mixed with each other.

6. Reagent preparation

1. Place all reagents in ice water for pre-cooling for at least 15 minutes until they have returned to solution state before use.
2. **Preparation of lysis working solution:** Before testing, prepare sufficient lysis working solution. For example, prepare 1020 µL of lysis working solution by mixing 1000 µL of lysis buffer, 10 µL of phosphatase inhibitor, and 10 µL of protease inhibitor. Mix well. Keep on ice during use, protect from light, and use the prepared solution within 20 minutes.

7. Operation procedures

Total Protein Extraction from Tissue

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

② Cut the tissue into small 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 pre-cooled phenylmethylsulfonyl fluoride.

④ Homogenize the tissue up and down in the ice bath approximately 30 times.

⑤ Transfer tissue homogenate to a 2 mL pre-cooled EP tube and place in ice bath for 15 minutes.

⑥ Centrifuge at 12,000×g at 4°C for 15 minutes. The supernatant is the total protein extract. Place on ice for detection.

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

2. Total Protein Extraction from Cells

a. Cell collection

Suspension cells: Transfer cell suspension to pre-cooled EP tubes, centrifuge at 4°C at 1,000×g for 10 minutes to remove supernatant. Wash once with PBS (0.01 M, pH 7.4) at 2-8°C, centrifuge at 4°C at 1,000×g for 10 minutes to remove supernatant, leaving cell pellet for use.

Adherent cells: Discard the culture medium and wash cells twice with PBS (0.01 M, pH 7.4) at 2-8°C. Scrape cells with a cell scraper, or treat with EDTA solution, detach cells with a pipette and transfer the cell suspension to a pre-cooled EP tube. Centrifuge at 4°C at 1,000×g for 10 minutes to remove supernatant. Wash once with PBS (0.01 M, pH 7.4) at 2-8°C, centrifuge at 4°C at 1,000×g for 10 minutes to remove supernatant, leaving cell pellet 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 pre-cooled phenylmethylsulfonyl fluoride.

② Place in ice bath for 15 minutes, vortex and mix every 5 minutes for 10 seconds each time.

③ Centrifuge at 12,000×g at 4°C for 15 minutes. The supernatant is the total protein extract, which should be placed on ice for detection.

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

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