Technical Bulletin 1/1.

Product Information

Total Protein Extraction Reagent

(Catalog Number: VTP-001) Size: 50 mL Stored at - °C

Description:
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The total protein extraction reagent is an all-in-one solution, including the complete protease/phosphatase inhibitors (I208052), designed for rapidly extracting intact, non-denatured total protein fragments from various cell types or tissues without freeze/thaw cycles or sonication, preparation for ELISA, EMSA, 1D and 2D electrophoresis, western blotting, TF-TF interaction arrays and other protein/DNA assays.

Protocol: (Keep reagent and cell/tissue samples on ice)

1. Method-1 (cells)

- Harvest cells (approximately 50ul packed cells or 1x 10e7 cells) into a clean 1.5mL tube from culturing wells/plates/flasks as usual and wash cells once with 1x ice-cold PBS/DPBS, centrifuge at 1,600 rpm for 8 minutes, aspirate liquids.
- Add 500ul ice-cold total protein extraction reagent and incubate on ice for 20 minutes. Vortex vigorously at highest speed for 15 seconds every 5 minutes.
- Centrifuge at 14,000xg for 10 minutes at 4◦C.
- Transfer the supernatant containing total protein into a new 1.5mL microcentrifuge tube. •

Determine the total protein concentration by spectrometers or BCA Assay.
Store all the extract aliquots at -80°C.

2. Method-2 (Tissues)

- Add 500ul ice-cold total protein extraction reagent into a clean 1.5ml mcrocentrifuge tube containing 0.2-0.5g frozen/ fresh tissues. (Note, chop tissues into small pieces using a clean razor blade may help to completely lysate tissues and increase the yields of total proteins).
- Homogenization: using a pre-chilled, clean Dounce homogenizer to homogenizate the tissues twice at speed 4 (moderate) speed for 20 seconds. **Or** using the pre-chilled Teflon pestle homogenizer to homogenize the tissues/cells 15-20 strokes on ice.
- Vortex vigorously and incubate on ice for 20 minutes. Vortex at highest speed for 15 seconds every 5 minutes.
- Centrifuge at 14,000xg for 10 minutes at 4◦C.
- Transfer the supernatant containing total protein into a new 1.5mL microcentrifuge tube. •

Determine the total protein concentration by spectrometers or BCA Assay.
Store all the extract aliquots at -80°C.

3. Method-3 (Cells)

- Directly add 500ul ice-cold total protein extraction reagent into the wells/plates/flasks containing the culturing cells.(Approximately 50ul packed cells or 1x 10e7 cells)
- Scrape all the cells into a clean 1.5ml tube and incubate on ice for 20 minutes. Vortex vigorously at highest speed for 15 seconds every 5 minutes.
- Centrifuge at 14,000xg for 10 minutes at 4◦C.
- Transfer the supernatant containing total protein into a new 1.5mL microcentrifuge tube. •

Determine the total protein concentration by spectrometers or BCA Assay.

- Store all the extract aliquots at -80°C.

Additional information:

- The nuclear protein markers: Lamin B (68kDa), LaminA/C (70 KDa) , P84, Histone H1 (33KDa), Histon H4(43KDa);
- The Cytoplasmic protein markers: GAPDH, anti-b-actin;
- The membrane protein markers: EGFR, Na+/K+ ATPase, anti-Sp1; The cytoskeleton protein markers: Vimentin.
- **Protease/Phosphatase Inhibitors (I208052)** supplied in DMSO, contains optimized AEBSF, Aprotinin, E64, Leupeptin, Pepstatin A, Sodium fluoride, Sodium orthovanadate and Sodium pyrophosphate.

Precautions and Disclaimer:

This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.