Total Protein Extraction Kit

INTRODUCTION

Total Protein Extraction; TPE kit contains ready to use buffers for extracting total protein from tissues, cell lines, bacteria, yeast and plant. The two-component protocol eliminates clump formation, protein loss and other related problems during total protein extraction procedure. TPE Buffer kit is based on optimized concentration of Tris and SDS and is suitable for running SDS-PAGE and other downstream applications.

ITEM(S) SUPPLIED Cat # 786-225

TPE Buffer-I	100ml
TPE Buffer-II	12ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store at 4°C upon arrival and is stable for 1 year.

ITEMS NEEDED BUT NOT SUPPLIED WITH THE KIT

Protease inhibitor cocktail (e.g. *G-Biosciences's Protease Arrest, Cat # 786-108*)

Preparation Before Use

I. Let the solution II come to room temperature before use.

II. OPTIONAL - Take an appropriate volume of TPE Buffer I, depending on your research needs, add protease inhibitor cocktail (Protease Arrest; *G-Biosciences Cat # 786-108*), reducing agents or any other reagents. Keep the buffer chilled in ice or refrigerator. Always prepare fresh solution before use.

III. Prepare a hot water bath, set the hot water bath to boiling temperature. Place a vortexer close to a boiling water bath.

WARNING- Wear protective gloves and eye protective glasses

Protocol for Total Protein Extraction from Animal Tissues:

1. Weigh an appropriate amount of tissue (e.g. 200mg – 250mg tissue).

2. Add 1.5-2.0ml of TPE Buffer-I for 200-250mg tissues. (Alternatively, for each 1g of tissue, use 7-8ml TPE Buffer-I). Transfer it to a grinder and grind the tissue until you get a homogeneous suspension.

   Warning *-Prevent heating during grinding of the tissue, keep in ice.*

3. Transfer the homogenate to a 15ml tube and add 1/9 volume of TPE Buffer-II (Alternatively, for each 100µl TPE Buffer-I, add 12µl TPE Buffer-II). Vortex it immediately for 30 seconds to achieve a complete mixing of homogenate with TPE Buffer-II.

4. Place the tube in a boiling hot water bath for 30 seconds. Remove the tube and vortex for 30 seconds. Repeat this heating and vortexing process (30 sec each) until you get a clear solution.

5. Keep the tube in a boiling water bath for another 5-10 minutes.

6. Centrifuge the tube for 5 minutes at 15, 000 x g, at 4°C for any tissue debris. Transfer the supernatant to another tube and the lysate is ready for use, which may be stored at -20°C to -70°C.

Protocol for Total Protein Extraction from Cell Lines and Bacteria:

1. Thaw the cell pellets on ice and tap the tube gently to resuspend the cell pellet.
2. Add appropriate volumes of previously prepared TPE Buffer-I to the cell pellet (e.g., for each 100µl cell pellet, use approx 500µl TPE Buffer-I). Vortex the tube for 1-2 minutes. Keep the tube on ice.
3. Add TPE Buffer-II, 1/9 of total cell suspension volume. Alternatively, for each 100µl TPE Buffer-I used add 12µl TPE Buffer-II. Mix it gently by taping the tube with finger. If needed vortex the tube for 30 seconds to achieve complete mixing,
4. Place the tube in the boiling hot water bath for 30 seconds. Remove the tube and vortex for 30 seconds. Repeat this heating and vortexing process until you have a clear solution.
5. Keep the tube in boiling water bath for another 10 minutes.
6. Centrifuge the tube for 5-6 minutes at 15, 000 x g, at room temp, to remove any debris present. Then transfer the supernatant to another tube and the lysate is ready for use. Lysate may be stored at -20°C to -70°C.

Protocol for Total Protein Extraction from Yeast:

1. Thaw the cell pellets on ice.
2. Add appropriate volumes of previously prepared TPE Buffer-I to the cell pellet (e.g., for each 100µl cell pellet, use approx 500µl TPE Buffer-I). Add 2-3 times pellet volume of 0.5mm glass beads. Keep the tube in ice water bath. Sonicate the suspension 10-12 times, 30 seconds each. Chill the tube in the ice water bath at least 30 seconds between sonication to prevent heating.
3. Transfer the homogeneous suspension to a new tube. Add TPE Buffer-II, 1/9 of total suspension volume. Mix it gently by taping the tube with finger. If needed vortex the tube for 30 seconds to achieve complete mixing,
4. Place the tube in the boiling hot water bath for 30 seconds. Remove the tube and vortex for 30 seconds. Repeat this heating and vortexing process until you have a clear solution.
5. Keep the tube in boiling water bath for another 10 minutes.
6. Centrifuge the tube for 5-6 minutes at 15, 000 x g, at room temp, to remove any debris present. Then transfer the supernatant to another tube and the lysate is ready for use. Lysate may be stored at -20°C to -70°C.

Protocol for Total Protein Extraction from Plant Tissues:

1. Weigh an appropriate amount of tissue (e.g., 0.5-1.0g tissue).
2. Add TPE Buffer-I (e.g., 1.0-2.0ml of TPE Buffer-I). Alternatively, for each 1gm of tissue, use 2ml TPE Buffer-I. Transfer it to a grinder and grind the tissue until you get a homogeneous.
   Warning *-Prevent heating during grinding of the tissue, keep in ice.*
3. Transfer the homogenate to a 15ml tube and add 1/9 volume of TPE Buffer-II. Vortex immediately for 30 sec to achieve a complete mixing of homogenate with TPE Buffer-II.
4. Place the tube in a boiling hot water bath for 30 seconds. Remove the tube and vortex for 30 seconds. Repeat this heating and vortexing process (30 sec each) until you get a clear solution.
5. Keep the tube in a boiling water bath for another 5-10 minutes.
6. Centrifuge the tube for 5 minutes at 15, 000 x g, at 4°C for any tissue debris. Transfer the supernatant to another tube and the lysate is ready for use. Lysate may be stored at -20°C to -70°C.

RELATED PRODUCTS

1. Protease Arrest (Cat # 786-108): *A cocktail of protease inhibitors for use during protein extraction and purification. Protease Arrest inhibits a broad spectrum of serine, cysteine and metalloproteases as well as calpains*

2. NI Protein Assay Kit (Cat # 786-005): *A protein assay that is free from interference of common laboratory agents including reducing agents, detergents, dyes, EDTA etc.*

3. PAGE Perfect (Cat # 786-123): *A kit for preparing sample for PAGE electrophoresis.*

4. RAPID-Stain *(Cat # 786-31): For staining protein in gels. RAPID-Stain only stains proteins, leaving clear background with high band visibility. Generally does not require de-staining.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*