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A Geno Technology, Inc. (USA) brand name

Total Protein Extraction Kit

(Cat. # 786-225)

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)

Description	Size
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.

ADDITIONAL ITEMS REQUIRED

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

PREPARATION BEFORE USE

- Warm TPE Buffer-II to room temperature before use.
- **OPTIONAL**: Add appropriate protease inhibitor cocktail, reducing agents or any other reagents to an appropriate volume of TPE Buffer I. Keep the buffer chilled on ice. Always prepare fresh solution before use.
- Prepare a hot water bath, set the hot water bath to boiling temperature.

PROTOCOL FOR TOTAL PROTEIN EXTRACTION FROM ANIMAL TISSUES

1. Add 800µl TPE Buffer-1 to every 100mg tissue to be lysed.
2. Transfer to a grinder or homogenizer and grind the tissue until an homogeneous suspension is achieved. Prepare on ice to avoid excess heating and protein degradation.
3. Transfer the homogenate to a 15ml tube and add 96µl TPE Buffer-II for every 100µl TPE Buffer-I used. Vortex immediately for 30 seconds.
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 until a clear solution is seen.
5. Incubate in the boiling water bath for an additional 10 minutes.
6. Centrifuge the tube for 5 minutes at 15, 000 x g at 4°C to remove any tissue debris. Transfer the supernatant to a clean tube. 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 500µl TPE Buffer-I for every 100µl cell pellet. Vortex the tube for 1-2 minutes and place on ice.
3. Add 60µl TPE Buffer-II for each 100µl TPE Buffer-I used. Mix gently by tapping the tube with finger. If needed vortex the tube for 30 seconds to achieve complete mixing,
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 until a clear solution is seen.
5. Incubate in the boiling water bath for an additional 10 minutes.
6. Centrifuge the tube for 5 minutes at 15, 000 x g at 4°C to remove any cell debris. Transfer the supernatant to a clean tube. The lysate is ready for use, which may be stored at -20°C to -70°C.

PROTOCOL FOR TOTAL PROTEIN EXTRACTION FROM YEAST

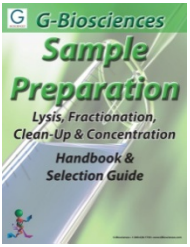
1. Thaw the yeast cell pellets on ice and tap the tube gently to resuspend the cell pellet.
2. Add 500µl TPE Buffer-I for every 100µl cell pellet. Add 2-3 times pellet volume of 0.5mm glass beads. Keep the tube in an ice water bath. Sonicate the suspension 10-12 times, 30 seconds each. Chill the tube in the ice water bath at least 30 seconds between sonication to prevent heating.
3. Add 60µl TPE Buffer-II for each 100µl TPE Buffer-I used. Mix gently by tapping the tube with finger. If needed vortex the tube for 30 seconds to achieve complete mixing,
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 until a clear solution is seen.
5. Incubate in the boiling water bath for an additional 10 minutes.
6. Centrifuge the tube for 5 minutes at 15, 000 x g at 4°C to remove any cell debris. Transfer the supernatant to a clean tube. The lysate is ready for use, which may be stored at -20°C to -70°C.

PROTOCOL FOR TOTAL PROTEIN EXTRACTION FROM PLANT TISSUES:

1. Add 2ml TPE Buffer-1 to every 1g plant tissue to be lysed.
2. Transfer to a grinder or homogenizer and grind the tissue until an homogeneous suspension is achieved. Prepare on ice to avoid excess heating and protein degradation.
3. Transfer the homogenate to a 15ml tube and add 120µl TPE Buffer-II for every 1ml TPE Buffer-I used. Vortex immediately for 30 seconds.
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 until a clear solution is seen.
5. Incubate in the boiling water bath for an additional 10 minutes.
6. Centrifuge the tube for 5 minutes at 15, 000 x g at 4°C to remove any tissue debris. Transfer the supernatant to a clean tube. The lysate is ready for use, which may be stored at -20°C to -70°C.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/

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