PROMETHEUS™
Protein Biology Products

Instruction Manual

Universal Protein Extraction Kit- with Protease Inhibitor Cocktail, General-EDTA Free

Catalog No.: 18-418 and 18-419

Introduction:

Our Universal Protein Extraction (UPE) kit has been designed for the extraction and solubilization of total proteins from different types of tissues and cells for denaturing proteomics applications. The kit is suitable for extracting total proteins from animal and plant tissues, bacteria, mammalian and yeast cells. The protein extraction solutions supplied in the kit contain Tris buffer, detergent SDS with other proprietary components and the extracted proteins are suitable for running SDS-PAGE, Western Blot and other downstream applications.

The kit is also supplied with a Protease Inhibitor Cocktail-EDTA Free at 100X concentration, containing AEBSF, Aprotinin, Bestatin, E64, Leupeptin and other proprietary component(s), optimized for inhibiting broad spectrum of proteases present in the extracted protein samples and is better than Roche and Sigma protease inhibitor cocktail. The kit components are stable for one year from the delivery date.

Kit Components

Name	**Cat. No. 18-418** (50 Preps)	**Cat. No. 18-419** (100 Preps)	**Storage* Temp**
UPE Solution-1	50 ml	100 ml	4°C
UPE Solution-2	8 ml	15 ml	4°C
Protease Inhibitor Cocktail [100X], General-EDTA Free	1 ml	2 ml	4°C

*The kit is shipped at ambient temperature. Upon receiving, store at the recommended temperature.

Preparation Before Use

Prepare fresh solution before use as below-

A. Thaw UPE Solution-2 (Do not Shake or Vortex) and Protease Inhibitor cocktail at room temp.
B. Add the supplied Protease Inhibitor Cocktail-EDTA Free [100X] to an appropriate volume of PE Solution-1 to give 1X concentration (e.g. 10 µl of supplied Protease Inhibitor Cocktail [100X] in 1 ml of lysis buffer or lysate) and if needed, up to 2X concentration can be used.
C. If the protein extraction buffer doesn't contain EDTA in it and your protein of interest is compatible with EDTA, 10 µl of 0.5M EDTA (not supplied) per ml sample can be added for the inhibition of metalloproteases at 1X concentration along with Protease Inhibitor Cocktail. Higher concentration of EDTA 20 µl to 30 µl of 0.5M EDTA per ml sample can be added, if needed.
D. Add reducing agent(s) and/or any other agent to an appropriate volume of UPE Solution-1. Keep UPE Solution-1 with inhibitor cocktail and other agents chilled on ice.
E. Get ready a hot boiling water bath for boiling the sample at the last step.

Total Protein Extraction from Animal Tissues

1. Add 800µl UPE Solution-1 to each 100mg tissue to be extracted for total proteins.
2. Transfer it to a grinder and grind the tissue until a homogeneous suspension is formed. Perform this step on ice to avoid protein degradation due to heat generation.
3. Transfer the homogenate to a 10ml tube with cap and add 100µl UPE Solution-2 and vortex it immediately for 30-45 seconds, otherwise clumps would form.
4. Place the 10ml tube with lysate in a boiling hot water bath for 30-45 seconds and vortex it every 15-20 seconds. Repeat the heating and vortexing process until a clear solution appears, only then proceed to the next step.
5. Incubate the tube in the boiling water bath for 10 minutes.
6. Centrifuge the tube for 5 minutes at 15,000 x g for any tissue debris to settle in the bottom.
7. Transfer the clear supernatant (animal tissue lysate) to a new tube and the protein extract is ready to use for downstream proteomics application or can be stored at -20°C (for short term 3-4 weeks) to -80°C (for long term, more than 4 weeks).

Total Protein Extraction from Plant Tissues

1. Add 1000µl UPE Solution-1 to each 500mg plant tissue to be extracted for total proteins.
2. Transfer it to a grinder and grind the tissue until a homogeneous suspension is formed. Perform this step on ice to avoid protein degradation due to heat generation.

3. Transfer the homogenate to a 10ml tube with cap and add 120µl UPE Solution-2 and vortex it immediately for 30-45 seconds, otherwise clumps would form.
4. Place the 10ml tube with lysate in a boiling hot water bath for 30-45 seconds and vortex it every 15-20 seconds. Repeat the heating and vortexing process until a clear solution appears, only then proceed to the next step.
5. Incubate the tube in the boiling water bath for 10 minutes.
6. Centrifuge the tube for 5 minutes at 15,000 x g for any tissue debris to settle in the bottom.
7. Transfer the clear supernatant (plant tissue lysate) to a new tube and the protein extract is ready to use for downstream proteomics application or can be stored at -20°C (for short term 3-4 weeks) to -80°C (for long term, more than 4 weeks).

Optional: *This optional step can be performed, if needed to remove the plant pigments and other substances from plant tissue lysate. Take 10ml chilled Acetone (at -20^{0}C for at least one hour) to a centrifuge tube and add 1ml of plant protein lysate into it, mix it gently and incubate the tube at -20^{0}C for 2 hours. Centrifuge the tube in a cold centrifuge (2-4^{0}C) at 15,000xg for 15 minutes, which would precipitate the protein, leaving impurities in the supernatant. Decant off the supernatant careful without dislodge the protein pellet. Invert the tube on a new blotting paper for about 10 seconds, without disturbing the pellet. Resuspend the protein pellet using the buffer as per the application, e.g. Sample Loading Buffer for SDS-PAGE, etc., and other buffers for downstream applications.*

Total Protein Extraction from Bacterial and Mammalian Cells

1. Thaw the bacterial or mammalian cell pellets on ice and resuspend the cell pellet by gently tapping the tube.
2. Add 500µl UPE Solution-1 for each 100µl cell pellet and vortex the tube for 1-2 minutes and place it on ice.
3. Add 60µl UPE Solution-2 and mix it by gently by tapping the tube. If needed, vortex the tube for an additional 25-30 seconds to achieve complete mixing,
4. Place the tube in a boiling hot water bath for 30-45 seconds and vortex it every 15-20 seconds. Repeat the heating and vortexing process until a clear solution appear, only then proceed to the next step.
5. Incubate the tube in a boiling water bath for 10 minutes.
6. Centrifuge the tube for 5 minutes at 15,000 x g at 4°C for any cell debris to settle in the bottom.
7. Transfer the clear supernatant (bacterial or mammalian cell lysate) to a new tube and the protein extract is ready to use for downstream proteomics application or can be stored at -20°C (for short term 3-4 weeks) to -80°C (for long term, more than 4 weeks).

Optional: *For Gram-positive bacteria, which contain a thick peptidoglycan cell wall, use lysozyme enzyme to first prepare the spheroplast or use the glass bead and sonication steps as in the Yeast Cells below, then move on to the protein extraction steps for maximum yield.*

Total Protein Extraction from Yeast Cells

1. Thaw the yeast cell pellets on ice and tap the tube gently to resuspend the cell pellet.
2. Add 500µl UPE Solution-1 for every 100µl yeast cell pellet. Add 2-3 times pellet volume of
0.5mm glass beads. Keep the tube in an ice water bath.
3. Sonicate the suspension 8-10 times, 30 seconds each burst and chill the tube in the ice water bath at least 30 seconds between sonication to prevent damage to proteins due to heat.
3. Add 60µl UPE Solution-2 and mix it gently by tapping the tube with finger. If needed, vortex the tube for additional 25-30 seconds to achieve complete mixing.
4. Place the tube in a boiling hot water bath for 30-45 seconds and vortex it every 15-20 seconds. Repeat the heating and vortexing process until a clear solution appear, only then proceed to the next step.
5. Incubate the tube in a boiling water bath for 10 minutes.
6. Centrifuge the tube for 5 minutes at 15,000 x g at 4°C for any cell debris to settle in the bottom.
7. Transfer the clear supernatant (yeast cell lysate) to a new tube and the protein extract is ready to use for downstream proteomics application or can be stored at -20°C (for short term 3-4 weeks) to -80°C (for long term, more than 4 weeks).
Optional: *Lyticase enzyme can be used to prepare the spheroplast, then move on to the other protein extraction steps.*

RELATED PRODUCTS:

1. **Protein Extraction Buffers/ Kits (Cat. No. 18-400, 18-402, 18-404, 18-406, 18-409, 18-411**
   For extracting proteins from Bacteria, Insects Cells, Mammalian cells, Tissues and Yeast samples.
2. **RIPA Lysis Buffer (18-415, 18-416 and 18-417:**
   For extracting proteins from different species samples.
3. **Protein Loading Buffer [2X], Cat. No. 20-309 and 20-310:**
   Non-reducing (20-309) and reducing (20-310) ready to use buffer for loading protein samples on to the gel. Premixed, just add an equal volume to your protein sample.