PROMETHEUS™
Protein Biology Products

Instruction Manual

Tissue Protein Extraction Buffer

Catalog No.: 18-409 & 18-410

Introduction:
Extraction of tissue proteins being an essential step in the process of protein sample preparation is critical to successful protein analysis. A successful tissue protein extraction requires efficient cell lysis and protein solubilization, while preserving protein integrity and immunoreactivity. The varying composition in different tissue samples implies different approaches for efficient and homogenous tissue protein extraction. Our Tissue Protein Extraction Buffer has been designed for use with different types of tissues, independent of mechanical extraction methods. A mild non-denaturing and easily dialyzable detergent is added to the solution that enhances solubilization of total proteins without compromising their functions, and presence of salt and other proprietary agents enable more efficient extraction from cellular compartments yielding homogeneous lysates. The extracted proteins are in their native state and compatible with enzyme assays, e.g. reporter gene expression assays (luciferase, beta-galactosidase, chloramphenicol acetyl transferase, CAT, alkaline phosphatase), protein kinase assays (PKA, PKC, tyrosine kinase), phosphatase assays (general phosphatases, tyrosine phosphatases), immunoassays (Western blots, ELISAs, RIAs, immunoprecipitation), Coomassie-Blue and silver staining, protein purification procedures, electrophoresis, folding studies, chromatographic studies, DNA-protein interaction assays (gel-shift assays), and many other downstream applications. The extraction buffer is also compatible with protease inhibitors, kinase and phosphatase inhibitors, and with BCA protein assays.

Items Supplied:

Name	Cat. No. 18-409	Cat. No. 18-410	Storage Condition*
Tissue Protein Extraction Buffer	250 ml	500 ml	4^0C

**Shipped at ambient temperature and upon receiving, store at 4^0C. The buffer is stable for one year, if stored and used as recommended.*

Additional Items Needed (Not Supplied):

- Appropriate tissue
- Protease inhibitors (Genesee Cat. No. 18-421) and/or phosphatase inhibitors
- 2 ml microcentrifuge tubes
- Tissue homogenizer
- Microcentrifuge

Important Information about the Product

1. All steps of protein lysis should be operated on ice or at 4 ˚C.
2. Use BCA Protein Assay kit (Genesee Cat. No. 18-440) to quantify extracted proteins.
   NOTE: *Bradford Protein Assay kit is not recommended.*

Protocol for Tissue Protein Extraction

Note: *Pre-chill an appropriate volume of Tissue Protein Extraction Buffer at 4^0C. If desired, add protease inhibitors and phosphatase inhibitors to the lysis buffer immediately before use.*

1. Place the fresh tissue into chilled PBS and rinse several times. Mince the tissue into small pieces.
2. Add Mammalian Tissue Protein Extraction Buffer to the tissue at 10:1. (i.e., add 10 ml chilled lysis buffer per gram of tissue.) Use a smaller volume of reagent if a more concentrated protein extract is required.
3. Homogenize the tissue for several minutes at high speed until no tissue chunks are left.

NOTE: *Tissue homogenization must be performed at 4^0C as the temperature of the lysate/buffer would rise during the process. Homogenizing the tissue with ~15-20 seconds of grinding and holding the homogenate in ice-cold bucket for 1-2 minutes would minimize the temperature rise in the lysate*

4. Incubate on ice for 30 minutes.
5. Centrifuge at ~14,000 x g for 10 minutes.
6. Carefully, transfer supernatant containing the tissue proteins to a new tube for further analysis and downstream application. ***NOTE:*** *Insoluble proteins will remain in the pellet that can be further analyzed.*

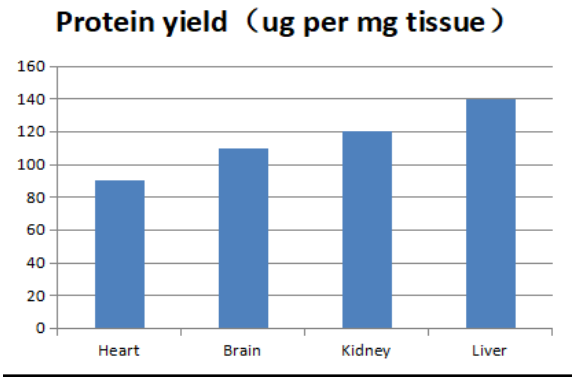

Figure 1: Protein yield with Tissue Protein Extraction Buffer. Tissue proteins were extracted from different mouse tissues using Tissue Protein Extraction Buffer protocol. The protein concentration of each lysate was estimated by BCA Protein Assay Kit.

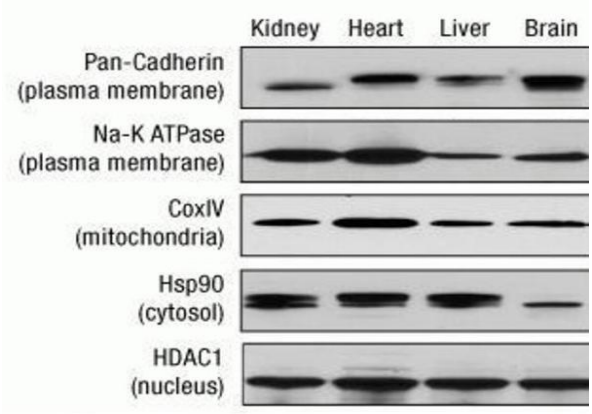

Figure 2: Protein identification, using Western Blot. The proteins were extracted from mouse tissues using Tissue Protein Extraction Buffer. Lysates (20µg) were separated by SDS-PAGE and transferred to a nitrocellulose membrane and probed using respective antibodies and chemiluminescent reagents.

TROUBLESHOOTING

Problem	Possible Cause	Solution
Protein yield is low.	Insufficient volume of Tissue Protein Extraction Buffer used	Add some more tissue protein extraction buffer
Tissue extract is too dilute	Extraction Buffer volume is not optimized and excess buffer was used	Use less extraction buffer or optimize the volume
Tissue extract is too concentrated	Extraction Buffer volume is not optimized and less buffer was used	Use more extraction buffer or dilute the cell extract with the buffer
Extracted Proteins are getting degraded	Proteolysis	Use Protease Inhibitor Cocktail (Genesee Cat. No. 18-421) and perform all lysis steps at 4^0C

Related Products

1. **BCA Protein Assay Kit (Cat. No. 18-440 & 18-441)**
   For protein estimation and is also compatible with proteins extracted with our Protein Extraction Buffers
2. **Protein Loading Buffer [2X], Cat. No. 20-309**
   Non-reducing ready to use buffer for loading protein samples on to the gel.
3. **Protein Loading Buffer [2X], Cat. No. 20-310**
   Reducing ready to use buffer for loading protein samples on to the gel.