Protocol

Tissue Cell Lysis Buffer

Catalog # GB-181

Introduction

Tissue Cell Lysis Buffer has been developed for extraction of total soluble protein from fresh or frozen animal tissues. Tissue Cell Lysis Buffer is based on an organic buffer, which utilizes a mild non-ionic detergent, and a proprietary combination of various salts and agents to enhance extraction and stability of proteins. Depending on the application, additional agents such as chelating agents, reducing agents and protease inhibitors may be added into Tissue Cell Lysis Buffer *(See Related Products for protease inhibitor cocktail- ProBlock Gold™)*. Tissue Cell Lysis Buffer reagent has been tested for use with a wide variety of animal tissues.

Tissue Cell Lysis Buffer is compatible with most applications, including enzyme assays, various chromatography procedures, electrophoresis, etc. The protein extract prepared with Tissue Cell Lysis Buffer may be used for most enzyme assays including reporter gene assays (e.g. ß-galactosidase, luciferase, chloramphenicol acetyl transferase), kinases (e.g., PKC, PKA, Tyrosine Kinase), and immunoassays (e.g., ELISA, Western blots, RIA).

Materials

- Tissue Cell Lysis Buffer (GoldBio Cat# GB-181)

Additional Items Needed

- Centrifuge
- Test tubes
- Incubator

Storage/Handling

Shipped at ambient temperature. Upon arrival, store it refrigerated at 4°C. If stored properly, it is stable for 1 year*.

Method

Preparation

Depending on applications, DTT (GoldBio Catalog # DTT10) and EDTA (GoldBio Catalog # E-210) may be added. Prepare an appropriate volume of the Tissue Cell Lysis Buffer use by adding DTT and EDTA both to a final concentration of 5mM. If the presence of a divalent metal ion is necessary for any application, do not add EDTA; instead, add an appropriate divalent salt to a final concentration of 5mM.

If the inhibition of protease activity is required, add a cocktail of protease inhibitors to prevent protease activities during the extraction procedure (see Related Products for protease inhibitor cocktail Protease Gold™).

Lysis of Cell Suspension

1. Weigh the tissue sample. For each gram of tissue used for extraction of proteins, use approximately 15-20 ml Tissue Cell Lysis Buffer. If there is need for preparing a more concentrated protein extract, the volume of the Tissue Cell Lysis Buffer added may be reduced by 20-30%.

2. Homogenize the tissue in the presence of the Tissue Cell Lysis Buffer. Make sure that the homogenization is performed with an efficient instrument (e.g., pestle-tube homogenizers, electrical blender or grinders, etc.). Homogenization should be performed at 4°C and during homogenization care must be taken to prevent the rise of temperature. As a safe practice, homogenize the tissue with brief bursts of actions (10-15 seconds) and between homogenization hold the homogenate in ice-cold bucket for 1-2 minutes.

3. Centrifuge the homogenate to pellet the tissue debris at 20,000 x g for 30 minutes at 4°C.

4. Collect the clear supernatant for further processing or analysis.

5. The debris may contain many nuclear and membrane bound proteins. Debris may be further extracted by adding appropriate detergents in the Tissue Cell Lysis Buffer

Associated Products

- ProBlock™ Gold (GoldBio Catalog # GB-108): A cocktail of protease inhibition for use during protein extraction and purification. ProBlock Gold™ inhibits a broad spectrum of serine, cysteine and metalloproteases as well as calpains.
- ProBlock™ Gold 2D (GoldBio Catalog # GB-109): A cocktail of protease inhibition for use during protein sample preparation for IEF/2D studies. ProBlock Gold™ inhibits a broad spectrum of serine, cysteine and metalloproteases as well as aspartic proteases and aminopeptidases.