Protocol

TD-P Revision 3.0

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Mammalian Cell Lysis Buffer

Introduction

Mammalian Cell Lysis Buffer has been developed for extraction of total soluble proteins from mammalian cultured cells. The Mammalian Cell Lysis Buffer is based on organic buffering agents, 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 Mammalian Cell Lysis Buffer (see Related Products for protease inhibitor *ProBlock Gold™*). Mammalian Cell Lysis Buffer reagent has been tested for use with a wide variety of mammalian cells. Mammalian Cell Lysis Buffer can be used for both suspension as well as adherent cells. The proprietary combination of this reagent provides a simple and versatile method for the extraction of proteins from mammalian cells.

Mammalian Cell Lysis Buffer is compatible with most applications, including enzyme assays, various chromatography procedures, electrophoresis, etc. The protein extract prepared with Mammalian 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, Tyrosin Kinase), and immunoassays (e.g., ELISA, Western blots, RIA).

Materials

- Mammalian Cell Lysis Buffer (GoldBio Catalog # GB-180)
- 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

Depending on applications, DTT (GoldBio Catalog # DTT10) and EDTA (GoldBio Catalog # E-210) may be added. Prepare an appropriate volume of the Mammalian 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.

Gold Biotechnology
St. Louis, MO
Ph: (800) 248-7609
Web: www.goldbio.com
Email: contactgoldbio86@goldbio.com

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. Pellet the cells by centrifugation at 200-500 x g for 5 minutes. Remove and discard the supernatant. For adherent cells, scrape or detach cells from the culture plate, centrifuge and discard the supernatant.

2. Wash the cell pellet once with 5-10 ml PBS.

3. Remove the supernatant and then resuspend the cells in 5-10 ml PBS.

4. Pellet the cells again by centrifugation at 200-500 x g for 5 minutes. Remove and discard the PBS wash.

5. Vortex and suspend the pellet in the remaining volume of PBS wash. Add Mammalian Cell Lysis Buffer and use a pipette to suspend the cell pellet until you have a homogeneous suspension. For each 10 ml of fully-grown suspension culture, add approximately 1 ml Mammalian Cell Lysis Buffer. Alternatively, add 1 ml Mammalian Cell Lysis Buffer lysis buffer for each 0.05 g of wet cell pellet. For making even more concentrated cell extract, the volume of Mammalian Cell Lysis Buffer added to the pellet may be reduced. In such cases, one freeze and thaw cycle will ensure complete lysis of the cells.

6. Incubate the suspension on ice for 15-30 minutes. Periodically shake or briefly vortex the suspension.

   Note: Freeze and thaw step is not necessary for lysis, however, one or two freeze and thaw cycle is not detrimental to the cell extract, and often ensures complete lysis.

7. Centrifuge the suspension at 20,000 x g for 30 minutes in a refrigerated centrifuge. Collect the clear suspension for downstream processing and analysis.

   Note: The cellular debris may contain some nuclear and membrane bound proteins, which may be further extracted with a variety of detergents.

Lysis of Adherent Mammalian Cells

1. Remove the culture medium from the adherent cells.

2. Wash the cells once with PBS to remove any residual growth medium. Remove the PBS wash.

3. Add an appropriate volume of the Mammalian Cell Lysis Buffer to cover the culture surface area. For example:
    a. Add 50-100 µl/well in 96 well plate
    b. Add 100-200 µl/well in 24 well plate
    c. Add 200-400 µl/well in 6 well plate
    d. Add 250-500 µl/well in 60 mm culture plate
    e. Add 500-1000 µl/well in 100 mm culture plate

4. Shake the culture plate gently for 10 minutes.

    Note: If a more concentrated cell lysate is required, the volume of the Mammalian Cell Lysis Buffer added to the culture plate may be reduced as appropriate. Subject the culture plate or well to one cycle of freeze and thaw. Shake gently for 10 minutes.

5. Lysate, including cellular debris may be used directly from the culture wells/plates. Alternatively, transfer the lysate to a centrifuge tube and centrifuge the lysate at 20,000 x g for 30 minutes. Collect the clear lysate for downstream processing and analysis.

    Note: (OPTIONAL) Add NaCl to a final concentration of 0.1M NaCl (use a 2-4M NaCl solution). Addition of NaCl generally improves performance of many immunoassays.

    Note: The cellular debris may contain some membrane bound protein, which may be further extracted with a variety of detergents.

Associated Products

- ProBlock™ Gold (Cat # 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 Mammalian (Cat # GB-331): A cocktail of protease inhibition for use during protein extraction and purification from mammalian cells and tissues. ProBlock Gold™ inhibits a broad spectrum of mammalian serine, cysteine and metalloproteases as well as aminopeptidases.