Instructions 28-9444-56 AA

Mammalian Protein Extraction Buffer

Introduction

Mammalian Protein Extraction Buffer has been developed for extraction of total soluble proteins from mammalian cultured cells. The Mammalian Protein Extraction Buffer is based on organic buffering agents, which utilize mild non-ionic detergents, 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 Protein Extraction Buffer (see Related Products for protease inhibition – Protease Inhibitor Mix).

Mammalian Protein Extraction Buffer reagent has been tested for use with a wide variety of mammalian cells. Mammalian Protein Extraction 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.

Compatibility

Mammalian Protein Extraction Buffer is compatible with most applications, including enzyme assays, various chromatography procedures, electrophoresis, etc. The protein extract prepared with Mammalian Protein Extraction Buffer may be used for most enzyme assays including reporter gene assays (e.g. β-galactosidase, luciferace, chloramphenicol acetyltransferase), kinases (e.g., PKC, PKA, Tyrosin Kinase), and immunoassays (e.g., ELISA, Western blots, RIA).

Items included

Name	Size
Mammalian Protein Extraction Buffer	500 ml

Storage condition

Shipped at ambient temperature. Upon arrival, store at 4–8°C. Stable for 1 year when stored and used as recommended.

Additional items

Centrifuge, test tubes, and incubator.

Instructions for use

Mammalian Protein Extraction Buffer is intended for research use only.

Preparation Before Use

Depending on applications, DTT and EDTA may be added. Prepare an appropriate volume of the Mammalian Protein Extraction Buffer for use by adding DTT and EDTA both to a final concentration of 5 mM. 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 5 mM.

Protease Inhibition

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 inhibition – Protease Inhibitor Mix).

Lysis of Cell Suspension

1 Pellet the cells by centrifugation at 3,000 × 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. Pellet the cells again by centrifugation. Remove and discard the PBS wash.

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

4 Use a pipette to suspend the cells until you have a homogeneous suspension. 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 cycles are not detrimental to the cell extract, and often ensures complete lysis.*

5 Centrifuge the suspension at 20,000 × 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. Remove the PBS wash.

3 Add an appropriate volume of the Mammalian Protein Extraction Buffer to cover the culture surface area.

For example: Add 50–100 µl/well in 96 well plate
Add 100–200 µl/well in 24 well plate
Add 200–400 µl/well in 6 well plate
Add 250–500 µl/well in 60 mm culture plate
Add 500–1000 µl/well in 100 mm culture plate

Shake the culture plate gently for 10 minutes.

Note: *If a more concentrated cell lysate is required, the volume of the Mammalian Protein Extraction 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.*

4 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 × g for 30 minutes. Collect the clear lysate for downstream processing and analysis.

Optional: Add NaCl to a final concentration of 0.1 M NaCl (use a 2–4 M 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.*

Order information

Re-ordering Information for Mammalian Protein Extraction Buffer:

Product	Quantity	Code No.
Mammalian Protein Extraction Buffer	1 × 500 ml	28-9412-79

Related products

Product	Quantity	Code No.
2D Quant Kit	500 assays	80-6483-56
Nuclease Mix	0.5 ml	80-6501-42
Protease Inhibitor Mix	1 ml	80-6501-23
SDS-PAGE Clean-Up Kit		80-6484-70
His SpinTrap™	50 × columns	28-4013-53
His MultiTrap™ HP	4 × 96-well plates	28-4009-89
His MultiTrap FF	4 × 96-well plate	28-4009-90
His GraviTrap™	10 × 1 ml columns	11-0033-99
HisTrap™ FF crude	5 × 1 ml	11-0004-58
PD-10 Desalting columns	30 × columns	17-0851-01
VivaSpin™ ultracentrifugation devices	multiple	

For more information on these and other related products, visit our web site at
www.gelifesciences.com or contact our technical dept.

For contact information for your local office, please visit,
www.gelifesciences.com/contact

www.gelifesciences.com/sampleprep

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue
P.O. Box 1327
Piscataway
NJ 08855-1327
USA

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