G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

RIPA Lysis & Extraction Buffer

For the Optimal Lysis of Adherent & Suspension Cells

(Cat. # 786-489, 786-490)

INTRODUCTION

G-Biosciences RIPA Lysis & Extraction Buffer is a highly reliable buffer for the lysis of adherent and suspension mammalian cells and subsequent release of cytoplasmic, membrane and nuclear proteins from adherent and suspension cultured mammalian cells. The RIPA Lysis & Extraction Buffer is fully compatible with many applications, including reporter assays, protein assays, immunoassays and other protein purification techniques.

The RIPA Lysis & Extraction Buffer can be used for the lysis of mammalian tissue.

ITEM(S) SUPPLIED

Description	Cat. # 786-489	Cat. # 786-490
RIPA Lysis & Extraction Buffer	100ml	500ml

25 mM Tris, 150 mM Sodium Chloride, 1% NP-40, 1% Sodium Deoxycholate, 0.1% SDS, pH 7.6

STORAGE CONDITIONS

Shipped at ambient temperature; upon arrival, store at RT. The product is stable for up to one year, if stored/used properly.

PREPARATION BEFORE USE

- RIPA Lysis & Extraction Buffer does not contain protease or phosphatase inhibitors to protect proteins following lysis. We recommend adding protease inhibitor cocktails, such as G-Biosciences ProteaseARREST™ (Cat. # 786-108) and PhosphataseARREST™ (Cat. # 786-450) to prevent proteolytic breakdown and maintain the protein's phosphorylation state.
- Pre-chill the RIPA Lysis & Extraction Buffer prior to use and added inhibitors immediately before use.

PROTOCOL FOR LYSIS OF ADHERENT CELLS

1. Remove the growth media from the cells and wash the cells twice with ice cold PBS.
2. Add 1ml ice-cold RIPA Lysis & Extraction Buffer to every 75cm^2 flask containing 5x10^6 mammalian cells.
3. Incubate on ice for 5-15 minutes with periodical pipetting.
4. Use a cell scraper to pool the cell lysate in the flask and transfer to a microcentrifuge tube.
5. Centrifuge at ~14,000xg for 15 minutes to pellet cell debris.
   NOTE: *Sonicate the pellet to increase protein yield for 30 seconds at 50% pulse.*

6. Transfer supernatant to a fresh tube for downstream applications.

PROTOCOL FOR LYSIS OF SUSPENSION CELLS

1. Centrifuge the cell suspension at 2,500xg for 5 minutes to collect the cells. Discard the supernatant.
2. Wash the cells twice in ice-cold PBS, pelleting the cells as before.
3. Add 1ml ice-cold RIPA Lysis & Extraction Buffer to every 40mg or ~$5x10^6$ or ~20µl wet cell pellet of mammalian cells. Pipette up and down to mix.
4. Incubate on ice for 5-15 minutes with periodical pipetting.
5. Centrifuge at ~14,000xg for 15 minutes to pellet cell debris.
   NOTE: *Sonicate the pellet to increase protein yield for 30 seconds at 50% pulse.*
6. Transfer supernatant to a fresh tube for downstream applications.

PROTOCOL FOR LYSIS OF TISSUE

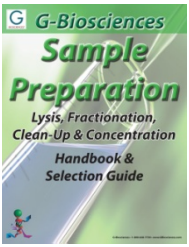
1. Add 1ml ice-cold RIPA Lysis & Extraction Buffer to every 100mg o mammalian tissue.
2. Sonicate the tissue on ice with ~5 x 30 second at 50% pulse. Allow sample to cool between each sonication burst. Ensure tissue is completely homogenized before proceeding.
3. Incubate on ice for 5-15 minutes with periodical pipetting.
4. Centrifuge at ~14,000xg for 15 minutes to pellet cell debris.
   Transfer supernatant to a fresh tube for downstream applications.

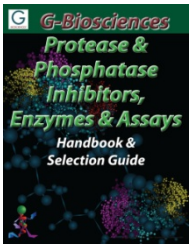
TROUBLESHOOTING

Issue	Possible Reason	Solution
Low protein yield	Some cells and tissue are more resistant to lysis	Introduce a sonication step.
		Extend incubation times.
		Ensure pellet is fully suspended in RIPA
Protein very dilute	Too much RIPA Lysis & Extraction Buffer used	Adjust volume of buffer used. i.e. Use 0.25-0.5ml/75cm^2 plate.
Protein degraded	No or insufficient protease inhibitors used	Use ProteaseARREST™ protease inhibitor cocktail
Low protein phosphorylation	Phosphatase activity in lysate	Use PhosphataseARREST™ phosphatase inhibitor cocktails
	Native protein is not phosphorylated or poorly phosphorylated	None

RELATED PRODUCTS

Download our Sample Preparation and Protease & Phosphatase Inhibitors, Enzymes & Assays Handbooks





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