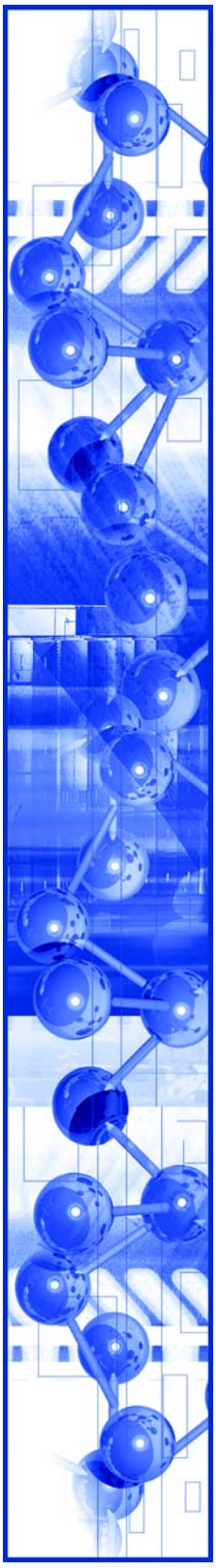

RIPA Cell Lysis Buffer

Code	Description	Size
N653-100ml	RIPA Cell Lysis Buffer	100mls

General Information:

AMRESCO's RIPA cell lysis buffer is used for total cell lysis of adherent or nonadherent cells in culture. RIPA Buffer enables the extraction of cytoplasmic, membrane and nuclear proteins and is compatible with many applications, including reporter assays, protein assays, immunoassays and protein purification. RIPA buffer is a more denaturing lysis buffer than NP-40. RIPA buffer contains the nonionic detergent NP-40, plus two ionic detergents sodium deoxycholate and SDS.

RIPA Buffer is a ready to use solution to which protease and phosphatase inhibitors may be added as needed. One ml of the RIPA Buffer is sufficient to lyse cells from one 100 mm culture dish or up to 5 x 10^7 cells of most adherent mammalian cell lines.

Storage/Stability:

Store product cold, 2-4˚C

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

Not Supplied:

PBS (Phosphate Buffered Saline) wash buffer

Protease Inhibitor Cocktails (if desired)

Phosphatase Inhibitor Cocktails (if desired)

Procedure:

As soon as lysis occurs, proteolysis, dephosphorylation and denaturation begin to occur. These events occur at a much faster rate at room temperature, than they do on ice. It is recommended to:

- Aspirate medium in the cold room.
- Perform all steps in the cold room.
- Use ice cold PBS for the washes.

Procedure for Lysis of Adherent Cultured Cells

All steps should be performed on ice.

1. Carefully remove (decant or aspirate) culture media from adherent cells.
2. Carefully wash cells twice with a volume of ice cold PBS equal to that of the culture media removed.

***Note**: RIPA Buffer does not contain any protease or phosphatase inhibitors. If desired, add cocktails to RIPA buffer immediately before applying to cells.*

3. Add cold RIPA buffer to the cells. Use 1 ml of RIPA lysis buffer per ~5x10^6 cells or 100mm cell culture dish.
4. Keep on ice 15 minutes, occasionally swirling the plate to keep solution evenly spread.
5. Use cell scraper to scrape off cells. Pass the cell lysate through pipette several times to form a homogeneous lysate.
6. Transfer lysate to a cold 1.5 ml microcentrifuge tube on ice.
7. Centrifuge the lysate at 14,000xg for 15 minutes at 4°C to separate the total protein (supernatant) from the cellular debris (pellet)
8. Transfer supernatant to a new cold tube for further analysis.
9. Total cell protein should be stored frozen at 20°C until needed.

Procedure for Lysis of Suspension Cultured Cells

1. Pellet the cells by centrifugation at 2,500x*g* for 5 minutes. Discard the supernatant.
2. Wash cell pellet twice in ice cold PBS. Pellet cells by centrifugation at 2,500xg for 5 minutes.
3. Add cold RIPA Buffer to the cell pellet. Use 1 ml of RIPA buffer per ~5 x10^6 cells. Pipette the mixture up and down to suspend the pellet.
4. Shake mixture gently for 15 minutes on ice. Centrifuge mixture at ~14,000x*g* for 15 minutes to pellet the cell debris.
5. Transfer supernatant to a new cold tube for further analysis.
6. The total cell protein should be stored frozen until needed.

Related Products

Code	Product
M221-1ml	Protease Inhibitor Cocktail, 100X
M222-1ml	Protease Inhibitor Cocktail 100X with EDTA
E504-100ml E504-500ml	Phosphate Buffered Saline, 1X Sterile Solution
N182-5x10ml	DMSO

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