RIPA Lysis Buffer

Code	Description	Size
N653-100ML	**RIPA Lysis Buffer**	100 mL

General Information

VWR Life Science AMRESCO's RIPA Lysis Buffer is used for total cell lysis and protein solubilization and is compatible with many applications, including reporter assays, protein assays, immunoassays and protein purification. Cytoplasmic, nuclear and membrane proteins are extracted with this single buffer. RIPA Lysis Buffer contains non-ionic detergent, Nonidet® P-40 Substitute, plus two ionic detergents sodium deoxycholate and SDS.

RIPA Lysis Buffer is a ready-to-use solution, which may be supplemented with protease and phosphatase inhibitors as needed. One milliliter of RIPA Lysis Buffer is sufficient to lyse cells up to 5 X 10^6 cells of most adherent and non-adherent cell lines.

Composition: 149 mM sodium chloride, 50 mM Tris pH 7.5, 0.1% sodium dodecyl sulfate, 1% Nonidet® P-40 Substitute, 0.5% deoxycholic acid sodium salt

Storage/Stability

Store cold (2 to 8°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Required Materials Not Supplied

Phosphate buffered saline (PBS)
Protease inhibitors (recommended)
Phosphatase inhibitors (recommended)
Cell scraper (for adherent cells)

Protocol/Procedure

Lysis of adherent cultured cells - *Perform all steps on ice.*

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

   Note: RIPA Lysis Buffer does not contain any protease inhibitors or phosphatase inhibitors. If desired, add inhibitors to RIPA Lysis Buffer immediately before applying to cells.
3. Add cold RIPA Lysis Buffer to the cells. Use 1 mLof RIPA Lysis Buffer for up to 5 x 10^6 cells.
4. Incubate on ice 15 minutes, occasionally swirling the plate to keep the surface evenly covered.
5. Use a cell scraper to remove lysate from the culture dish. Pass the cell lysate through a pipette several times to form a homogenous lysate.
6. Transfer lysate to a cold 1.5 mL microcentrifuge tube on ice.
7. Centrifuge the lysate at 14,000 x *g* for 15 minutes at 4°C to separate the total protein (supernatant) from the cellular debris (pellet).
8. Transfer the supernatant to a new 1.5 mL microcentrifuge tube on ice.
9. Total protein can be used immediately or stored frozen at -20°C until needed. Prepare small aliquots to avoid repeated freeze-thaw cycles, which may degrade the sample.

Lysis of suspension cultured cells - *Perform all steps on ice.*

1. Pellet cells by centrifugation at 2,500 x *g* for 5 minutes at 4°C. Discard the supernatant.
2. Wash the cell pellet twice in cold PBS using a volume equal to the culture media removed in step 1. Pellet the cells by centrifugation at 2,500 x *g* for 5 minutes at 4°C.
3. To the cell pellet obtained in step 3, add 1 mL of cold RIPA Cell Lysis Buffer for up to 5 x 10^6 cells. Pipette the mixture up and down to resuspend the pellet.
4. Shake the mixture gently for 15 minutes on ice.

5. Centrifuge the mixture at 14,000 x *g* for 15 minutes at 4°C to pellet the cell debris.
6. Transfer the supernatant containing total protein to a new cold 1.5 mL microcentrifuge tube.
7. Total protein can be used immediately or stored frozen at -20°C until needed. Prepare small aliquots to avoid repeated freeze-thaw cycles, which may degrade the sample.

Frequently Asked Questions

Questions	Answers
Why are my samples degraded?	Samples underwent proteolysis. Use protease inhibitors and keep sample on ice at all times.
Why is the concentration of my protein so low?	1. Cells were not completely lysed. Resuspend cells completely in RIPA Cell Lysis Buffer and increase incubation time on ice. Brief sonication at 50% puls may also increase yield. 2. Too few cells per volume of RIPA Cell Lysis Buffer were used. Increase number of cells or decrease volume of RIPA Cell Lysis Buffer.

For Technical Support

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