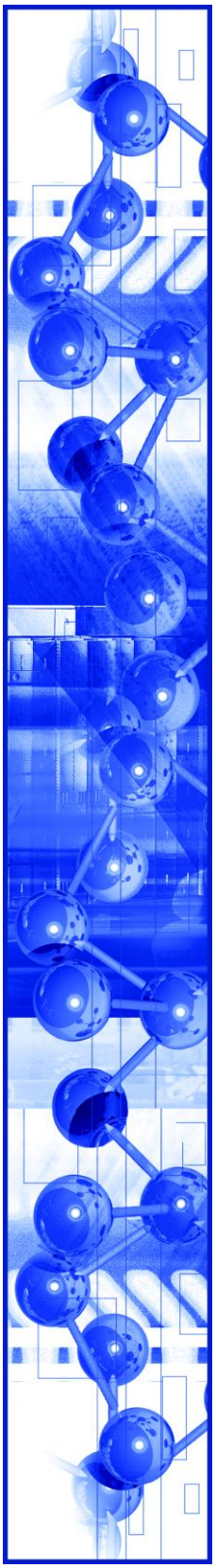

Hypotonic Lysis Buffer

Code	Description	Size
M334-100ML	Hypotonic Lysis Buffer	100ML
M334-30ML	Hypotonic Lysis Buffer	30ML

AMRESCO's Hypotonic Lysis Buffer is a detergent-free, ready-to-use solution for the isolation of cytoplasmic proteins from cultured mammalian cells. Cell lysis and subsequent isolation of the crude cytoplasmic fraction by a simple centrifugation step is complete in less than 30 minutes.

The protocol is compatible with a variety of downstream applications including Western Blotting and ELISA procedures as well as protein assays which are sensitive to the presence of detergents in the lysis buffer.

Storage/Stability:

Hypotonic Lysis Buffer is stable for 1 year when stored at 2-8°C

Application Disclaimer

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

Procedure:

Reagents not supplied:
PBS
Protease inhibitor cocktail

➥**Note:** All procedures should be performed on ice in a cold room with ice cold reagents to reduce proteolysis, dephosphorylation and denaturation.

Prior to beginning procedure:

1. Protease Inhibitors or Inhibitor Cocktails should be added to Hypotonic Lysis Buffer just prior to lysis.
2. Protocol below is optimized for $1x10^6$ or $5x10^6$ cells.

Protocol:

Cell Washing:

1. Transfer cells from tissue culture flask to an appropriate-sized tube.
2. Centrifuge at 2,000 rpm, for 5 minutes at 4˚C.
3. Decant the media and resuspend the pelleted cells in 10 ml ice cold PBS.
4. Centrifuge at 2,000 rpm for 5 minutes at 4˚C.
5. Decant the PBS supernatant and resuspend the pellet in 1 ml ice cold PBS. Transfer the resuspended pellet to a microcentrifuge tube.
6. Centrifuge 1 minute at 2,000 rpm, 4˚C.
7. Decant the PBS supernatant.

Cell Lysis:

8. Resuspend the cell pellet in 400ul ($5x10^6$ cells) or 800 ul ($1x10^6$ cells) of ice cold Hypotonic Lysis Buffer.
9. Incubate on ice for 15 minutes to allow cells to swell.
10. Disrupt the cells by homogenization with 10-20 strokes with a Dounce homogenizer.
11. Centrifuge 30 sec at 9,000 rpm at 4˚C.

➥**Note**: After the centrifugation in step 11, the cytoplasmic proteins are suspended in the supernatant while cell nuclei can be found in the pellet.

Cytoplasmic Proteins:

12. Transfer the supernatant containing **cytoplasmic proteins** from step 11 to a new microfuge tube.
13. (Optional) Centrifuge the cytoplasmic protein sample from step 11 at 14,000xg for 15 minutes at 4˚C.
14. Save the supernatant that contains the cytoplasmic proteins in a new microcentrifuge tube.
15. Store frozen until needed. Cryoprotectants may be added prior to freezing to preserve activity.

Related Products

Code	Product
M221-1ml	Protease Inhibitor Cocktail 100X, General Use
M222-1ml	Protease Inhibitor Cocktail 100X, General Use with EDTA
M250-1ml	Protease Inhibitor Cocktail Mammalian
E504-100ml E504-500ml	Phosphate Buffered Saline (PBS), 1X Sterile Solution
M329-10ml	Total Cell Protein Lysis Buffer
M330-kit	Cytoplasmic-Nuclear Protein Enrichment Kit
M328-30ml	Mitochondrial Protein isolation Buffer

Corporate Headquarters
AMRESCO LLC.
6681 Cochran Road
Solon, OH 44139 USA
Tel: (440) 349-1199
Toll free: 1-800-448-4442 (US & Canada)
Fax: (440) 349-2441
e-mail: info@amresco-inc.com

To Order (USA & Canada)
www.amresco-inc.com
Toll free: 1-800-448-4442
Toll free fax: 1-800-326-3733
Fax: (440) 349-3255
e-mail: dom-sales@amresco-inc.com

International Orders:
Tel: (440) 349-1199
Fax: (440) 349-2441
e-mail: int-sales@amresco-inc.com

Technical Support
Toll free: 1-800-610-2789 (USA & Canada)
Fax: (440) 349-0235
e-mail: techinquiry@amresco-inc.com