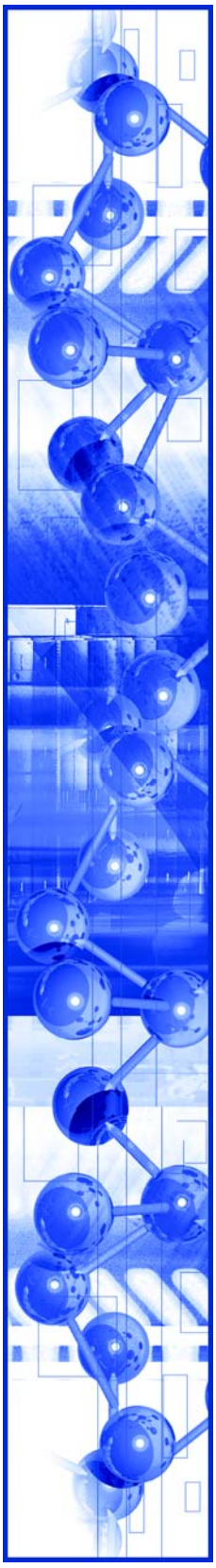

Mitochondrial Protein Isolation Buffer

Code	Description	Size
M328-30ML	Mitochondrial Isolation Kit *For Tissue Culture Cells* Includes sufficient buffer for 20 mitochondrial protein isolations	30 mls

General Information:

AMRESCO's Mitochondrial Protein Isolation Buffer is a convenient one-buffer solution for extraction of protein from mitochondrial fractions derived from cultured mammalian cells. The simple, scalable extraction procedure requires less than an hour and involves no toxic chemicals or ultracentrifugation steps.

Following cell lysis, the cytostolic fraction is separated by centrifugation from an enriched mitochondrial fraction. This separation allows the less complex set of mitochondrial proteins to be analyzed in a single sample and increases the likelihood of detecting low-abundance proteins. The mitochondrial protein fraction can be used to perform Western blotting, ELISA or other assays.

Storage/Stability:

Store product cold, 2-8˚C

Application Disclaimer

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

Customer-Supplied Items:

1cc syringe, 26 ½ G needle

1X ice cold PBS

Centrifuge tubes

Procedure:

All steps should be performed on ice in a cold room with ice cold reagents to reduce proteolysis, dephosphorylation and denaturation.

Prepare sufficient volume (~3 ml for 1X10^6 cells) of Mitochondrial Protein Isolation Buffer (including Protease Inhibitor).

Add protease inhibitor cocktail to the Mitochondrial Protein Isolation Buffer so that the final concentration of inhibitors is 1X.

Mitochondrial Protein Isolation Protocol:

1. Transfer cells from tissue culture flask to an appropriate-sized tube (~ 1x10^6 cells).
2. Centrifuge at 2,000 rpm, for 5 minutes.
3. Resuspend the pellet in 10ml cold 1X PBS and spin at 2,000rpm at 4°C for 5 minutes.
4. Remove the supernatant and resuspend the pellet in 1 ml ice cold 1X PBS. Transfer the resuspended cells to a 1.5 ml microcentrifuge tube. Spin at 2,000rpm, 4°C for 5 min.
5. Resuspend the pellet in the Mitochondrial Protein Isolation Buffer (including Protease Inhibitor).
6. Homogenize cells on ice by passage in a 1cc syringe with a 26 ½ G needle, twenty times (20x).
7. Centrifuge x 1000g at 4°C for 10 minutes.
8. Collect and transfer the supernatant to a fresh 1.5 ml tube. Discard the pellet (which contains whole cells and nuclei).
9. Centrifuge the collected supernatant at 14,000xg for 15 minutes at 4°C.
10. Collect and transfer the supernatant into a new tube labeled *cytosolic proteins*.
11. Resuspend the pellet (which contains mitochondrial proteins) in 1 ml Mitochondrial Protein Isolation Buffer.
12. Spin at 14,000xg for 1 min at 4°C. Discard supernatant.
13. Resuspend pellet in 40ul of Mitochondrial Protein Isolation Buffer per 1x10^6 cells starting material. Store the mitochondrial fraction protein lysate frozen until needed.

Related Products

Code	
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	Phosphate Buffered Saline (PBS),
E504-500ml	1X Sterile Solution
N655-50ml	SeraFree™ Cryopreservation Media (RPMI)
N655-6x5ml	
N673-50ml	SeraFree™ DMEM Cryopreservation Media
N673-6x5ml	
0260-25g	Trypsin 1:300
0260-50g	
N182-5x10ml	DMSO, Ultra Pure Grade
K952-100ml	Penicillin/Streptomycin, 100X *Tissue Culture Grade*

Corporate Headquarters
AMRESCO Inc.
30175 Solon Industrial Pkwy.
Solon, OH 44139 USA
Tel: (440) 349-1199
Toll free: 1-800-829-2805 (US & Canada)
Fax: (440) 349-2441
e-mail: info@amresco-inc.com

www.interchim.com
interchim

211 bis Avenue Kennedy - BP 1140
03103 Montluçon - France
33 (0) 4 70 03 88 55
Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com

Agence Paris - Normandie
33 (0) 1 41 32 34 40
Fax 33 (0) 1 47 91 23 90
e-mail interchim.paris@interchim.com

www.amresco-inc.com