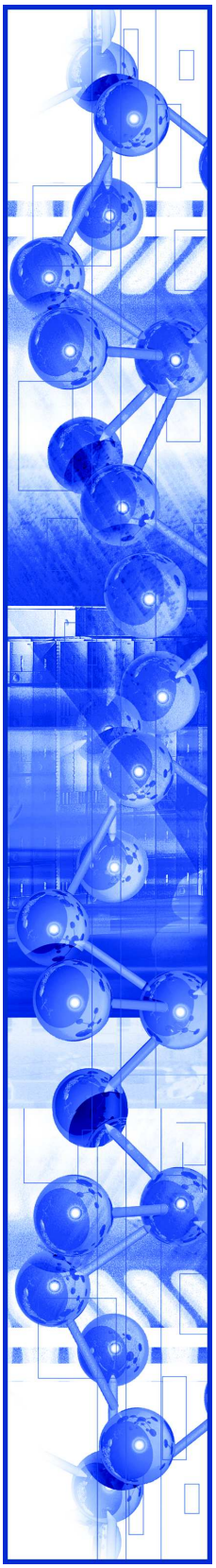


Cytoplasmic & Nuclear Protein Enrichment Kit

Code	Description	Size
M330-KIT	Cytoplasmic & Nuclear Protein Enrichment Kit *For Tissue Culture Cells* *Includes sufficient materials for 20 extractions of 1 x 10^6 cells:* M331-1ML CN Fractionation Buffer 1 (1.0 ml) M332-1.5ML CN Fractionation Buffer 2 (1.5 ml) M333-30ML CN Fractionation Buffer 3 (30.0 ml) E109-0.1ML Nonidet® P-40 Substitute (0.1 ml)	Kit

General Information:

AMRESCO's Cytoplasmic & Nuclear Protein Enrichment Kit provides a convenient procedure for the isolation of proteins from both the cytoplasmic and nuclear fractions of tissue culture cells. The separation of fractions reduces the complexity of each set of proteins and may increase the relative abundance of low level proteins.

The protocol separates the cytoplasm from the nucleus by a simple centrifugation step after cell lysis in a non-ionic detergent. Proteins from each fraction are then recovered from their respective fractions during subsequent fractionation steps. The entire procedure can be performed in under 1.5 hours. The procedure is completely scalable.

Storage/Stability:

Store product at 2-8℃.

Application Disclaimer

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

Procedure

Not supplied:

Ice cold 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) Add 0.9ml deionized water to E109-0.1ml Nonidet® P-40 Substitute, to make 1ml of 10% NP-40 Substitute solution. The 10% solution should be stored cold and used for all subsequent procedures.

2) Add Protease Inhibitor Cocktail to each volume of buffer used in the current working procedure so that the final concentration is 1X.

3) Protocol below is for $1x10^6$ or $5x10^6$ cells

Cytoplasmic / Nuclear Protein Enrichment 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. Aspirate the media and resuspend the pelleted cells in 10 mls ice cold PBS.
4. Centrifuge at 2,000 rpm for 5 minutes at 4°C.
5. Aspirate the PBS supernatant, and resuspend the pellet in 1ml ice cold PBS. Transfer the resuspended pellet to a microcentrifuge tube.
6. Centrifuge 1 minute at 2,000rpm, 4°C
7. Remove the PBS supernatant.

Cell Lysing:

8. Resuspend the cell pellet in 400ul ($5x10^6$ cells) or 80ul ($1x10^6$ cells) of ice cold CN Fractionation Buffer 1 (Cytoplasmic Lysis Buffer). Incubate on ice for 15 minutes to allow cells to swell.
9. Add 25ul ($5x10^6$ cells) or 5ul ($1x10^6$ cells) 10% NP-40 substitute and vortex for 10 seconds.
10. Centrifuge 30 sec at 9,000rpm at 4°C . (Quick Spin)

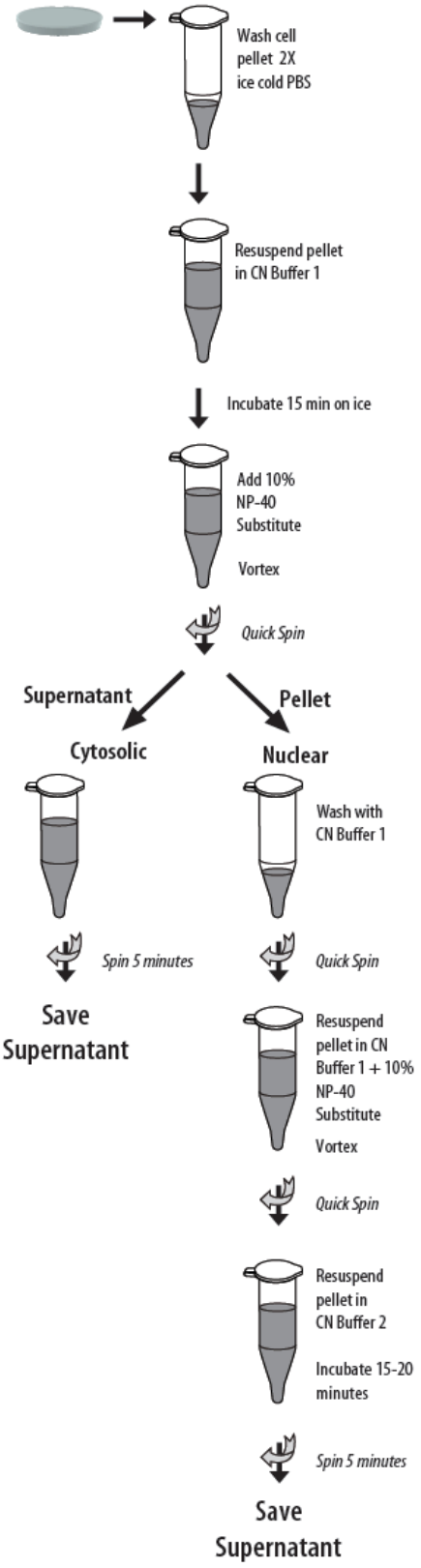
Note: After the centrifugation in step 10, the **supernatant contains the cytoplasmic proteins**, and the **pellet contains the nuclear proteins.**

Cytoplasmic Proteins:

11. Transfer the supernatant after step 10 containing **cytoplasmic proteins** to a new microcentrifuge tube. Add 0.11 volumes ice cold CN Fractionation Buffer 3 (Cytoplasmic Extraction Buffer) and mix well.
12. Centrifuge the cytoplasmic protein sample from step 11 at 14,000xg for 15 minutes at 4°C.
13. Save the supernatant that contains the cytoplasmic proteins in a new microcentrifuge tube.
14. Store frozen until needed.

Nuclear Proteins:

15. To the nuclear pellet from step 10, add 500ul ($5x10^6$ cells) or 100ul ($1x10^6$ cells) ice cold CN Fractionation Buffer 1. Centrifuge 30 seconds at 9,000 rpm, 4°C (Quick Spin).
16. Discard the supernatant. To the nuclear pellet add 500ul ($5x10^6$ cells) or 100ul ($1x10^6$ cells) ice cold CN Fractionation Buffer 1 plus 20ul ($5x10^6$ cells) or 4ul ($1x10^6$ cells) 10% NP-40 substitute. Vortex for 10 seconds.
17. Centrifuge the nuclear protein sample for 30 seconds at 9,000rpm, 4°C (quick spin).
18. Discard the supernatant. Resuspend the pellet in 50ul ($5x10^6$ cells) or 10ul ($1x10^6$ cells) CN Fractionation Buffer 2 (Nuclear Lysis Buffer) and shake at 4°C for 15-20 minutes.
19. Centrifuge the nuclear protein sample at 14,000xg for 5 minutes at 4°C.
20. Save the supernatant containing the nuclear proteins in new microcentrifuge tube.
21. Store frozen until needed.



Frequently Asked Questions

Questions	Answers
Why did I obtain low protein concentrations?	1. Incomplete cell lysis. Increase volume of buffers used. 2. Incomplete mixing. Vortex thoroughly to resuspend cells and homogenize samples. 3. Proteolytic degradation. Use protease inhibitor cocktail and keep samples on ice. Centrifuge at 4˚C.
Why is the nuclear protein yield low?	1. Incomplete isolation of nuclei. Increase centrifugation time at Step 10. 2. Inadequate dispersal of pellet in Step 16. Increase buffer and/or vortexing.
Why is there cross-contamination of cytoplasmic and nuclear fractions?	1. Incomplete cell lysis. Increase buffer volumes, vortexing and/or incubation times in buffers. 2. Inadequate removal of cytoplasmic extract. Transfer entire cytoplasmic extract at Step 11. Increase washing of nuclear pellet by repeating Step 15.

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
N655-50ML N655-6X5ML	SeraFree™ Cryopreservation Media (RPMI)
N673-50ML N673-6X5ML	SeraFree™ DMEM Cryopreservation Media
0260-25G 0260-50G	Trypsin 1:300
N182-5X10ML	DMSO, Ultra Pure Grade
K952-100ML	Penicillin/Streptomycin, 100X *Tissue Culture Grade*

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