Cytoplasmic / Nuclear Protein Enrichment Kit

Code	Description	Size
M330-KIT	**Cytoplasmic / Nuclear Protein Enrichment Kit**	20 preps from 1 x 10^6 cells

General Information

VWR Life Science 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 less than 1.5 hours. The procedure is completely scalable.

- Obtain distinct nuclear and cytoplasmic fractions in under 90 minutes
- For use with adherent or suspension culture cells
- Fractions compatible with downstream Western blotting

Storage/Stability

Store cold (2 to 8°C).

Product Use Limitations

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

Materials Supplied

M331-30ML CN Fractionation Buffer 1
M332-1.5ML CN Fractionation Buffer 2
M333-1ML CN Fractionation Buffer 3
E109-0.1ML Nonidet® P-40 Substitute

Required Materials Not Supplied

Ice-cold PBS
Protease Inhibitor Cocktail

Protocol/Procedure

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

Prior to beginning procedure

1. Add 0.9 mL deionized water to E109-0.1ML Nonidet® P-40 Substitute, to make 1 mL 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.

Cytoplasmic / Nuclear Protein Enrichment Procedure for 5 x 10^6 or 1 x 10^6 cells

Cell Washing

1. Transfer cells from tissue culture flask to an appropriate-sized centrifuge tube.
2. Centrifuge at 447 x *g* for 5 minutes at 4°C.
3. Aspirate the media and re-suspend the pelleted cells in 10 mL ice-cold PBS.
4. Centrifuge at 447 x *g* for 5 minutes at 4°C.
5. Aspirate the PBS supernatant and re-suspend the pellet in 1 mL ice-cold PBS. Transfer the re-suspended pellet to a microcentrifuge tube.
6. Centrifuge at 447 x *g* for 1 minute at 4°C.
7. Remove the PBS supernatant.

Cell Lysing

8. Re-suspend the cell pellet in 400 µL (5 x 10^6 cells) or 80 µL (1 x 10^6 cells) of ice-cold CN Fractionation Buffer 1 (Cytoplasmic Lysis Buffer). Incubate on ice for 15 minutes to allow cells to swell.
9. Add 25 µL (5 x 10^6 cells) or 5 µL (1 x 10^6 cells) 10% NP-40 Substitute solution and vortex for 10 seconds.
10. Centrifuge at 9,056 x *g* for 30 seconds at 4°C.

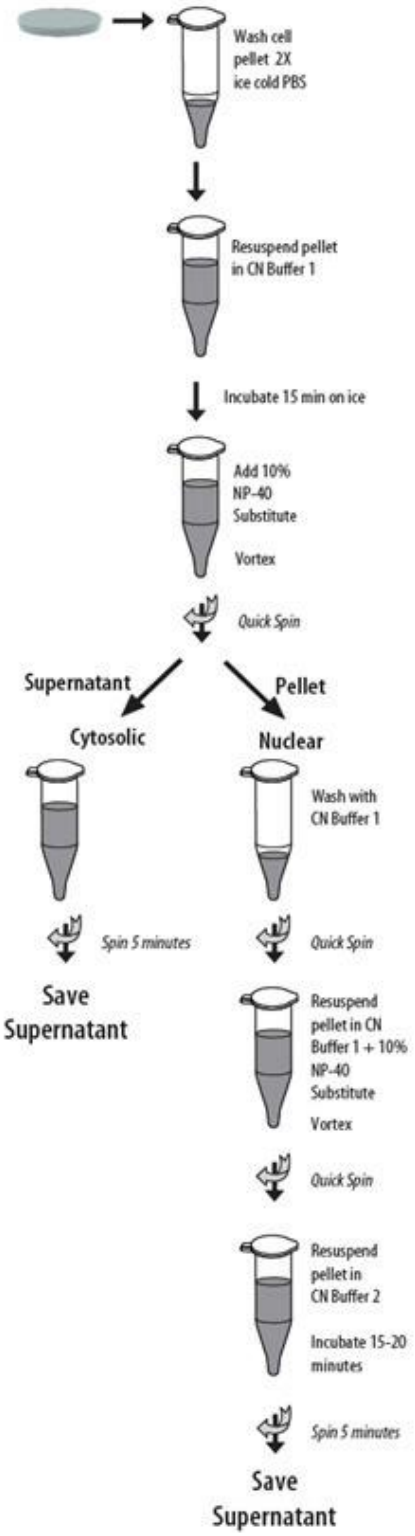
Note: After the centrifugation in step 10, the supernatant contains the cytoplasmic proteins, and the pellet contains the nuclear proteins.

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 21,913 x *g* 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 500 µL (5 x 10^6 cells) or 100 µL (1 x 10^6 cells) ice-cold CN Fractionation Buffer 1. Centrifuge at 9,056 x *g* for 30 seconds at 4°C.
16. Discard the supernatant. To the nuclear pellet add 500 µL (5 x 10^6 cells) or 100 µL (1 x 10^6 cells) ice-cold CN Fractionation Buffer 1 plus 20 µL (5 x 10^6 cells) or 4 µL (1 x 10^6 cells) 10% NP-40 Substitute solution. Vortex for 10 seconds.
17. Centrifuge the nuclear protein sample at 9,056 x *g* for 30 seconds at 4°C.
18. Discard the supernatant. Re-suspend the pellet in 50 µL (5 x 10^6 cells) or 10 µL (1 x 10^6cells) CN Fractionation Buffer 2 (Nuclear Lysis Buffer) and shake at 4°C for 15 – 20 minutes.
19. Centrifuge the nuclear protein sample at 21,913 x *g* for 5 minutes at 4°C.
20. Save the supernatant containing the nuclear proteins in new microcentrifuge tube.
21. Store frozen until needed.


Wash cell pellet 2X ice cold PBS
Resuspend pellet in CN Buffer 1
Incubate 15 min on ice
Add 10% NP-40 Substitute
Vortex
Quick Spin
Supernatant
Pellet
Cytosolic
Nuclear
Wash with CN Buffer 1
Spin 5 minutes
Quick Spin
Save Supernatant
Resuspend pellet in CN Buffer 1 + 10% NP-40 Substitute
Vortex
Quick Spin
Resuspend pellet in CN Buffer 2
Incubate 15-20 minutes
Spin 5 minutes
Save Supernatant

Frequently Asked Questions

Problem	Cause	Solution
Low protein concentrations	Incomplete cell lysis	Increase the volume of buffers used
	Incomplete mixing	Vortex thoroughly to re-suspend cells and homogenize samples
	Proteolytic degradation	Use a protease inhibitor cocktail in all buffers and keeps samples on ice.
Low nuclear protein yield	Incomplete isolation of nuclei	Increase the centrifugation time at Step 10.
	Inadequate dispersal of pellet in Step 16	Increase the buffer volume used and/or mixing time.
Cross-contamination of cytoplasmic and nuclear fractions	Incomplete cell lysis	Increase the volume of buffers used and/or the mixing and incubation times.
	Inadequate removal of cytoplasmic extract	Transfer the entire cytoplasmic extract at Step 11. Increase the washing of the nuclear pellet by repeating Step 15

For Technical Support

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