Protocol: Cytosolic and Membrane Fractionation

Buffer components

Buffer 1

	Concentration
HEPES (pH 7.4)	50 mM
NaCl	150 mM
Saponin	0.05%
PMSF	1 mM
Leupeptin	5 µg/ml
Aprotinin	2 µg/ml
Pepstatin A	1 µg/ml

Prepare just prior to use and place on ice.

Buffer 2

	Concentration
HEPES (pH 7.4)	50 mM
NaCl	150 mM
IGEPAL® CA-630 solution	1%
PMSF	1 mM
Leupeptin	5 µg/ml
Aprotinin	2 µg/ml
Pepstatin A	1 µg/ml

Prepare just prior to use and place on ice.

Buffer usage

Recommended volumes of Buffer 1 and Buffer 2.

Buffer	Cell number	Volume (µl)
Buffer 1	5×10^7~1×10^8	300-1000
	1×10^5	100-200
Buffer 2	5×10^7~1×10^8	500-1500
	1×10^5	100-300

Note:

1. Buffer volumes may vary based on cell type (i.e., cell size) and should be optimized by the user.
2. If total protein concentrations of the fractions are found to be low, please adjust buffer volumes accordingly.

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Protocol

Cytosolic fraction

1. Remove dishes or flasks from incubator. Collect cells in a centrifuge tube.
2. Count the cells and transfer the desired number of cells to a new centrifuge tube.
3. Centrifuge at room temperature for 5 min (200xg), discard the supernatant, and gently resuspend the cells in 1X PBS.
4. Centrifuge at room temperature for 5 min (200xg), then remove the wash.
5. Repeat 1X PBS resuspension and centrifugation. Be sure to remove all of the wash, leaving only the cell pellet.
6. Add the appropriate volume of Buffer 1 to the cell pellet, then gently resuspend the cells. Avoid foam formation.
7. Transfer the cell lysate to a new centrifuge tube.
8. Place the tube on ice for 10 min.
9. Centrifuge at 4°C for 5 min (2000xg), then transfer the supernatant (which is the cytosolic fraction) into another centrifuge tube on ice.
10. Confirm that the cytosolic fraction has been completely removed from the tube. Keep the pellet to obtain the membrane fraction in the following steps.

Membrane fraction

11. Add the appropriate volume of Buffer 2 to the pellet and gently resuspend.
12. Place the tube on ice for 30 min.
13. Centrifuge at 4°C for 5 min (7000xg).
14. Transfer the supernatant (which is the membrane fraction) to a new centrifuge tube on ice.

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