

## Reagents

### Subcellular fractionation buffer:

	<u>Stocks</u>	<u>50 ml 1X solution</u>
250 mM Sucrose	-	4.28 g
20 mM HEPES (7.4)	1 M	1 ml
10 mM KCl	-	0.0373 g
1.5 mM MgCl <sub>2</sub>	1 M	75 µl
1 mM EDTA	0.5 M	100 µl
1 mM EGTA	0.5 M	100 µl

At Time of Use: Take 10 ml and add the following:

1 mM DTT	1M	10 µl
PI Cocktail (III)		50 µl

## Procedure

Note: All centrifugations should be done at 4°C.

1. Lyse cells on 10 cm plates using 500 µl of buffer. Scrape plates immediately and place in 1.5 ml Eppendorf tube.
2. Then pass lysate through a 25 Ga needle 10 times using a 1 ml syringe.
3. Leave on ice for 20 minutes.
4. Centrifuge out the Nuclear Pellet (P1) at 720 G (3000 rpm) for 5 minutes. The nuclear pellet should be washed X1 by adding 500 µl of fractionation buffer again. Disperse the pellet with a pipette and pass through a 25 G needle 10X.; centrifuge again at 3000 rpm for 10 minutes. Remove the wash buffer, and resuspend the nuclear pellet in the nuclear buffer (standard lysis buffer with 10% glycerol and 0.1% SDS added). Sonicate the nuclear pellet briefly (3 seconds) on ice at a power setting of 2-continuous.
5. Remove the supernatant and place in a fresh labeled Eppendorf tube.
6. Centrifuge the supernatant again at 8000 rpm (10,000 G). Remove the supernatant again. This is the cytosolic and membrane fraction.
7. If the mitochondrial fraction is desired, save the pellet from #6, was as with the nuclear pellet and resuspend in the same buffer as above.
8. For a membrane fraction: centrifuge the supernatant in an ultracentrifuge. Centrifuge at 40,000 rpm (100,000G) for 1 hour. Wash this pellet as by adding 400 µl of the fractionation buffer to the pellet. Resuspend with by pipetting. Use a 25 G needle as above. Then re-centrifuge for 45 min. Resuspend the membrane pellet in the same buffer as used for the nuclei.
9. Optional: Concentrate the supernatant by centrifuging through the Filter Unit. This should concentrate the cytosol down to approximately 50-75 µl.

### Protocol provided by:

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