PROTOCOL FOR TISSUE SUBCELLULAR FRACTIONATION

Homogeneization buffer: (HB)	25 mM TrisHCl pH 7.4 to 7.6	(use stock 1M TrisHCl)
	320 mM Sucrose	(use stock 2M sucrose)
	1X protease inhibitors	(5 mM EDTA optional)
	1X phosphatase inhibitors type I and II	(optional)

This buffer (without protease and phosphatase inhibitors) must be filtered and stored at 4ºC or frozen in aliquots.

IMPORTANT: AS SOON AS THE TISSUE IS OUT OF THE FREEZER, ALL FOLLOWING STEPS MUST BE PERFORMED ON ICE OR AT 4°C!! PROTEINS WILL START DEGRADING IMMEDIATELY AFTER CELL RUPTURE!

IMPORTANT: THIS PROTOCOL GENERATES A TOTAL HOMOGENATE THAT CAN BE USED TO SEPARATE SOLUBLE AND MEMBRANE-BOUND PROTEINS. THIS PROTOCOL DOES NOT GENERATE A TISSUE LYSATE.

Full protocol (yields Th, P1, P2, P3 and S3):

1. Weight amount of tissue to be processed while it is still frozen. For small amounts (<150 mg) weight tissue directly in a pre-weighted 1.5 or 2.0 ml eppendorf tube
2. Add 10 weight-volumes of HB to the tissue and let tissue thaw on ice (e.g., for 1g tissue use 10ml HB)
3. Homogenize tissue in a Potter-Elvehjem tissue grinder for 1 minute using a medium drill speed or ~20 strokes with the Pestle (work on ice or in the cold room!)

(BE CAREFUL WITH THIS ITEM! THE PRESSURE AGAINST THE WALLS MAY CRACK THE GLASS)

4. Alternatively, if the tissue amount is very small, homogenize directly inside the eppendorf tube using a conical pestle and rotatory movements for 1 minute.
5. If desired, remove up to 1/10 total volume of tissue homogenate (Th) and prepare for PAGE or downstream processing. If frozen, aliquot in small aliquots (Th samples CANNOT be frozen and thawed multiple times)
6. Centrifuge samples at 800g x 10 min.
7. Carefully remove the supernatant (S1) without disturbing the nuclear pellet (P1). DO NOT DECANT, USE A FINE PASTEUR PIPETTE INSTEAD!.
8. OPTIONAL STEP: For less cross-contamination of P1, leave S1 on ice while resuspending P1 in 5vol of HB. Centrifuge this suspension again and separate P1' and S1'. Pool S1 and S1' for the next steps.
9. Centrifuge S1 (and S1') at 9000g x 20 min.
10. Carefully remove the supernatant (S2) without disturbing the mitochondrial pellet (P2). DO NOT DECANT, USE A FINE PASTEUR PIPETTE.

11. OPTIONAL STEP: For less cross-contamination of P2, leave S2 on ice while resuspending P2 in 5vol of HB. Centrifuge this suspension again and separate P2' and S2'. Pool S2 and S2' for the next steps.
12. Centrifuge S2 (and S2') at 100,000g x 30 min (check that the tubes can withstand this centrifugal force. If not, use at least 50,000g and double the time)
13. Remove the supernatant (S3, cytosol) from the plasma membrane pellet (P3). The supernatant may have a thin layer of floating myelin and other lipids (may be separated as an independent sample or pooled with S3).
14. Resuspend the final pellets P1, P2 and P3 in small volumes of HB (P3 is usually very small). Alternatively, resuspend them in 25mM TrisHCl (pH 7.4) for further processing without sucrose or in 25 mM TrisHCl (pH 7.4) with 1 % w/v CHAPS (or other suitable detergent) to make soluble extracts from the pellets. If using detergents, P1 may not resuspend well due to the high proportion of DNA and insoluble matrix components.
15. Measure protein concentration in all the samples and prepare for SDS-PAGE or any necessary downstream processing.

Simple protocol (yields Th, P1, P2 and S2):

1. Weight amount of tissue to be processed while it is still frozen. For small amounts (<150 mg) weight tissue directly in a pre-weighted 1.5 or 2.0 ml eppendorf tube
2. Add 10 weight-volumes of HB to the tissue and let tissue thaw on ice (e.g., for 1g tissue use 10ml HB)
3. Homogenize tissue in a Potter-Elvehjem tissue grinder (on ice or in the cold room!) for 1 minute using a medium drill speed or ~20 strokes with the Pestle.
4. Alternatively, if the tissue amount is very small, homogenize directly inside the eppendorf tube using a conical pestle and rotatory movements for 1 minute.
5. If desired, remove up to 1/10 total volume of tissue homogenate (Th) and prepare for PAGE or downstream processing. If frozen, aliquot in small aliquots (Th samples cannot be frozen and thawed multiple times)
6. Centrifuge samples at 900g x 10 min.
7. Carefully remove the supernatant (S1) without disturbing the nuclear pellet (P1). DO NOT DECANT, USE A FINE PASTEUR PIPETTE INSTEAD!.
8. Centrifuge S1 at 30,000g x 120 min (minicentrifuge) or 100,000g x 30 min (ultracentrifuge)
9. Remove the supernatant (S2, soluble fraction) from the full particulate pellet (P2).
10. Resuspend the final pellets P1 and P2 in small volumes 25mM TrisHCl (pH 7.4).
11. Measure protein concentration in all the samples and prepare for SDS-PAGE or any necessary downstream processing.