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PROTOCOL FOR MYELIN ENRICHMENT/ISOLATION

1. Homogenize 5 g CNS tissue in 100 ml of cold 20 mM TrisHCl (pH 7.4) containing 0.32 M sucrose and protease inhibitors.

2. Using 6 centrifuge tubes (35ml capacity), dispense 16 ml of sample on top of 16 ml 0.83M sucrose in each tube. Centrifuge at 75,000g for 30 min.

3. Collect the crude myelin at the interface between the sucrose layers.

4. Disperse the crude myelin in 200ml of 20 mM TrisHCl to reduce sucrose concentration. Homogenize well. Centrifuge at 75,000g for 15 min. Discard SN.

5. Resuspend the pellets in 200 ml of 20mM TrisHCl and centrifuge at 12,000g x 15min. Discard SN.

6. Resuspend again the pellets in 200 ml of 20mM TrisHCl and centrifuge at 12,000g x 15min. Discard SN. The pellet contains a **crude myelin fraction.**

7. Resuspend all the crude myelin pellets in 72ml of 0.3 M non-buffered sucrose. Dispense 12 ml of sample on top of 15ml of 0.83M sucrose in 6 x 35ml centrifuge tubes. Centrifuge again at 75,000g x 30min.

8. Collect the myelin at the interface between the sucrose layers.

9. Disperse the crude myelin in 200ml of 20 mM TrisHCl to reduce sucrose concentration. Homogenize well. Centrifuge at 75,000g for 15 min. Discard SN.

10. Resuspend the pellets in 200 ml of 20mM TrisHCl and centrifuge at 12,000g x 15min. Discard SN.

11. Resuspend again the pellets in 200 ml of 20mM TrisHCl and centrifuge at 12,000g x 15min. Discard SN. The pellet contains a **purified myelin fraction.**

12. Resuspend the pellet in ddH_2O, measure protein concentration, aliquot and store at -80C.