Fractionation of synaptosomes

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ABSTRACT

This protocol details a step by step method to prepare pure fractions of synaptosomes for biochemical analysis.

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1 Pre-chill the homogeniser and buffers on ice. Weigh the tissue

2 Add 4x volume of ice-cold homogenizing buffer to glass homogenizers

3 Apply 12 strokes of even tension with the homogeniser rod

4 Transfer to 1.5ml tube (original tube)

5 Determine which tissue has the smallest volume and use that volume for all samples

6 Centrifuge at 1,000g for 10mins

7 Transfer the supernatant into fresh 1.5ml tube and spin at 12,500g for 15mins

8 Discard supernatant carefully

9 Resuspend pellet in 1ml of H-buffer, transfer to fresh glass homogenizer and mash for 6 strokes even tension

10 In polypropylene/ultra-clear tube add 5ml of 1.2M sucrose

11 Slowly and steadily add in 5ml of 0.8M sucrose to create clear/distinct gradient

12 Overlay sample on top of gradient

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13 Weigh sucrose gradient tubes (add H-buffer if weight is off by more than 0.05g)

14 Centrifuge with slow accleration and zero brake in 4 degrees at 23,600g for 70 mins in a SW41 rotor

15 Using syringe, extract synaptosome layer between 0.8M and 1.2M sucrose and transfer into fresh 1.5ml tubes

16 Plate sample with appropriate dilutions into 24-well plate (dilute with H-buffer or PBS)

17 Spin in 4°C, max speed ~4,000 rpm for 20mins

18 Remove liquid and fix in 4% PFA for 20mins on rocker/shaker for 20mins

19 Wash x2~3 with PNBS

20 Either cover and place in fridge or move directly to immunofluorescence protocol

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