

**PSD fractionation from mouse brain protocol**  
**(adapted from Morgan Sheng's lab protocol)**  
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**Notes: You will need 4 cortices (or appx. 800 mg tissue) per genotype for this prep. DO NOT freeze tissue prior to performing fractionation; freezing will break membranes and cross-contaminate your fractions. All solutions MUST contain protease inhibitors (Roche Complete protease inhibitors + EDTA (Cat no. 1836145 (60 tab)/1697498 (20 tab)); dissolve 1 tablet in 500  $\mu$ L dH<sub>2</sub>O for 100x stock, or put 1 tablet in 50 mL solution). Phosphatase inhibitors are optional; I use Calbiochem's cocktail Set 1 (Cat no. 524624).**

The day before:

1. Prepare solutions A through E, plus 0.85M, 1.0M, and 1.2M sucrose solutions (see bottom of page) and store at 4°C.

The day of the prep:

1. Chill Dounce homogenizers (1 per 2 cortices) each containing 2.5 mL solution A on ice.
2. Dissect 2 cortices on ice and place in 1 homogenizer; homogenize slowly with 12 strokes of Teflon pestle. \*Remember to save some homogenate for analysis of PSD enrichment.
3. Transfer to 15 mL conical tube and spin at 1400 x g for 10 min at 4°C [Sorvall RT7 centrifuge w/ RTH-250 rotor in tissue culture room, 2600 rpm].
4. Collect supernatant in a separate conical tube on ice, and add 1 mL solution A to each pellet, resuspending pellet with pipet.
5. Transfer pellet mixture to Dounce homogenizer, and homogenize with 3 strokes.
6. Transfer homogenate back to conical tube and spin at 710 x g for 10 min at 4°C [Sorvall RT7 w/RTH-250 rotor, 1800 rpm].
7. Discard pellet, and combine supernatant with supernatant from step 4 above.
8. Spin combined supernatants at 13,800 x g for 10 min at 4°C [Sorvall RC5C+ w/ SLA-600TC rotor + 15 mL white conical adaptors, 9200 rpm].
9. Discard supernatant and resuspend pellet in 1 mL of solution B (per 2 cortices), and homogenize 6 strokes w/ Dounce homogenizer.
10. Take a Beckman polyallomer 13 mL tube (14 x 89 mm) and carefully pour 3 mL each of the following solutions in this order: 1.2M sucrose, 1.0M sucrose, and 0.85M sucrose. Store the prepared sucrose gradient columns on ice.
11. Slowly layer homogenate from step 9 on top of sucrose gradient, combining 4 cortices per column (ie, 2 mL homogenate per column).
12. Spin sucrose gradients at 82,500 x g for 2 hr at 4°C [Beckman LS-M ultracentrifuge HIM 7<sup>th</sup> floor w/ SW41 Ti rotor, 26,000 rpm].
13. Using a needle and syringe, slowly pull synaptosome fraction from interface between 1.0M and 1.2M sucrose layers. Note: Be very careful!! Puncture the tube slowly, and

have tape ready to close the hole when you've finished collecting the fraction. Puncture the tube under the band (fraction) to be collected, and place your needle at the bottom of the band with the open side of the needle facing up. FYI: the band on top of the 0.85M sucrose layer is the myelinated fraction; the band between the 0.85M and 1.0M sucrose layers is the ER/Golgi fraction; and the pellet is enriched for mitochondria and lysosomes.

14. Dilute the synaptosomal fraction to 5 mL w/ solution B and add 5 mL of solution C; incubate on ice 15 min.
15. IF you have only one sample: transfer 1.67 mL (1/6 of 10 mL) to each of six 4-mL micro-ultracentrifuge tubes (13mm x 51 mm); if you have 2 samples, transfer 1.67 mL to one 4-mL tube, and split the rest into 2 or 3 tubes. Spin at 32, 800 x g for 20 min at 4°C [Beckman micro-ultracentrifuge w/ TLA 100.3 rotor, 28, 200 rpm].
16. Discard supernatant, and resuspend the small pellet (from 1.67 mL tube) in 150 µL solution D. This is PSD I.
17. To the remaining pellets, resuspend in a total of 1 mL solution B.
18. To 0.4 mL of resuspension from step 17, add 1 volume (0.4 mL) of solution C, place on ice 15 min, and spin at 201, 800 x g for 1 hr at 4°C [TLA100.3 rotor= 70,000 rpm; TLA100.2= 75,200 rpm]. Resuspend pellet in 100 µL solution D containing 0.3% SDS (30 µL 10% SDS + 970 µL sol'n D); this is PSD II.
19. To the remaining 0.6 mL of resuspension from step 17, add 1 volume (0.6 mL) of solution E, place on ice 10 min, and spin at 201,800 x g for 1 hr at 4°C. Resuspend pellet in 100 µL solution D. This is PSD III.

### **Solutions**

**A: 0.32 M sucrose, 1 mM NaHCO<sub>3</sub>, 1 mM MgCl<sub>2</sub>, 0.5 mM CaCl<sub>2</sub>**

**B: 0.32 M sucrose, 1 mM NaHCO<sub>3</sub>**

**C: 12 mM Tris pH 8, 1% Triton X-100**

**D: 40 mM Tris pH 8**

**E: 12 mM Tris pH 8, 3% Sarkosyl**

**0.85 M sucrose/ dH<sub>2</sub>O**

**1.0 M sucrose/ dH<sub>2</sub>O**

**1.2 M sucrose/ dH<sub>2</sub>O**

### Ultracentrifuge tubes

For sucrose gradients: 13 mL Polyallomer tubes, 14 x 89 mm (Beckman # 331372)

For micro-ultracentrifuge: 4 mL Polycarbonate tubes, 13 x 51 mm (Beckman # 349622) [use TLA100.3 rotor]

Also 2.5 mL Polyallomer tubes, 11 x 34 mm (Beckman # 347287) [TLA100.2 rotor]

### Stock solutions

1M NaHCO<sub>3</sub>: (MW 84.01) 21.0 gm per 250 mL

1M CaCl<sub>2</sub>: (CaCl<sub>2</sub>\* 2H<sub>2</sub>O, MW 147.0) 36.75 gm per 250 mL

1M MgCl<sub>2</sub>: (MgCl<sub>2</sub>\* 6H<sub>2</sub>O, MW 203.3) 50.83 gm per 250 mL

To make solutions--

0.85 M sucrose: (MW 342.3) 14.55 gm per 50 mL

1.0 M sucrose: 17.12 gm per 50 mL

1.2 M sucrose: 20.54 gm per 50 mL

Solution A: (per 50 mL) 5.48 gm sucrose, 50  $\mu$ L 1M NaHCO<sub>3</sub>, 50  $\mu$ L 1M MgCl<sub>2</sub>, 25  $\mu$ L CaCl<sub>2</sub>

Solution B: (per 50 mL) 5.48 gm sucrose, 50  $\mu$ L 1M NaHCO<sub>3</sub>

Solution C: (per 50 mL) 0.6 mL 1M Tris pH 8, 0.5 mL Triton X-100

Solution D: (per 50 mL) 2 mL 1M Tris pH 8

Solution E: (per 50 mL) 0.6 mL 1M Tris pH 8, 1.5 gm N-laurylsarcosine