

Brain Membranes Preparation

To prevent proteolysis during the procedure, all the procedure should be performed on ice, and all the buffers and the rotors should be precooled. The following protease inhibitors cocktail should be used with all the buffers:

Pepstatin A (1 microgram/ml), leupeptin (1 microgram/ml), aprotinin (1 microgram/ml), Pefabloc SC (0.2 mM), benzamidine (0.1 mg/ml), and calpain inhibitors I and II (8 microgram/ml each).

Homogenize the brain in 320 mM sucrose in glass homogenizer.

Centrifuge the homogenate at 2000 g for 2-10 min.

Centrifuge the supernatant at $\sim 100000g$ for 1 h. Resuspend the pellet in the same solution, detect the protein content by Bradford, and store the aliquots at -70°C .

Pefabloc SC is a trademark for 4-(2-aminoethyl)-benzenesulfonyl fluoride, a water-stable analog of PMSF. It can be ordered from Merck under this trademark (Cat. # 124839), or from Sigma under the name AEBSF (Cat # A8456). Calpain inhibitors I and II are, respectively, N-Acetyl-Leu-Leu-Norleucinal (Cat. # A6185 in Sigma), and N-Acetyl-Leu-Leu-Methioninal (Cat. # A6060).