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Heart Membrane 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).

Buffer: 4 mM HEPES, pH 7.0; 320 mM sucrose + protease inhibitors.

1. Freeze the fresh heart sample in liquid nitrogen.
2. Make a powder by using a mortar and pestle, adding liquid nitrogen.
3. Add 10 ml buffer / 1 g heart powder.
4. Homogenize a powder in glass homogenizer.
5. Centrifuge at 2000 g 10 min.
6. Take a supernatant; add the new portion of a buffer to the pellet and re-homogenize it.
7. Centrifuge the re-homogenized pellet at 2000 g 10 min.
8. Pool the supernatants, centrifuge them at 100, 000 g 1 h.
9. Resuspend the pellet in the same buffer (0.5 ml / g heart).
10. Measure the protein concentration by Bradford, make aliquots and store at -70 ^{o}C.