Before Starting

- Prepare High salt buffer, Carbonate buffer, Wash buffer, Protease inhibitors.
- Instruments you need: ULTRA-TURRAX Dispenser T10 basis S25 (IKA) (or other dispensers); Ultracentrifuge.

1. Thaw 20–50 mg of tissue or 15CM dish cells in 1 mL of high salt buffer.

   **High salt buffer:** To impair of ionic bonds. 2 M NaCl, 10 mM HEPES–NaOH, pH 7.4, and 1 mM EDTA. Store at 4°C.

2. Homogenize tissues using an IKA Ultra Turbax blender at maximum speed (~25,000 rpm) for 30 s.

3. Ultracentrifuge the suspension in Beckman MLA 130 at 100,000 g for 10 min. The tubes should be balanced to a difference less than 50 mg.

4. Discard the supernatant and homogenize pellets in 1 mL of carbonate buffer as in step 2.

   **Carbonate buffer:** To allow efficient removal of soluble proteins. 1 M Na2CO3 and 1 mM EDTA, pH 11.3. Store at 4°C.
5 Incubate for 30 min with gentle mixing.

6 Collect the non-soluble material by centrifugation as in step 3 (If the content of integral membrane proteins in the purified membranes is not at least 30–40% of total identified proteins, steps 4–6 can be repeated two to three times).

7 Discard supernatant and resuspend pellets in wash buffer as in step 2. 

Wash buffer: To melt non-integral membrane proteins. 4 M urea, 100 mM NaCl, 10 mM HEPES-NaOH, pH 7.4, and 1 mM EDTA.

8 Collect the crude membranes by centrifugation as in step 3.