Crude Membrane protein extraction from tissues

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Abstract

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Before start

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

Protocol

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

NOTES
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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.

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

Step 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.

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

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Carbonate buffer: To allow efficient removal of soluble proteins1 M Na2CO3 and 1 mM EDTA, pH 11.3. Store at 4°C.

Step 5.
Incubate for 30 min with gentle mixing.

Step 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).

Step 7.

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

NOTES

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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.

Step 8.

Collect the crude membranes by centrifugation as in step 3 .

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