Plate T84 cells at high density overnight

Swell cells in hypo-osmotic buffer for 3 hours

Remove buffer and collect cells by scraping

Homogenize with a 22G needle followed by a 25 G needle

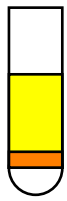
Add protease inhibitors to total cell homogenate

Remove cell fragments and nuclei by centrifugation at 30,000 xg for 10 min

Centrifuge supernatant at 100,000 xg for 60 min against a saturated sucrose pad

Collect 100,000 xg membranes and discard supernatant

Wash 100,000 xg membranes with distilled water and centrifuge at 100,000 xg



Solubilize washed 100,000 xg membranes with a final concentration of 2% CHAPS

Add primary antibodies and incubate overnight at 4 degrees Celsius with agitation

Remove precipitates by centrifugation at 10,000 xg for 30 min, collect supernatant

Add Protein A sepharose beads and incubate overnight at 4 degrees Celsius

Elution immuno-complexes with specific peptide antigen at room temperature

(1) Analyze purity of the complex by negative staining electron microscopy
(2) Analyze specificity of the complex by western blotting of tight junction proteins
(3) Visualize protein complexes by silver stain SDS gel
(4) Concentrate sample, run SDS gel, mass spectroscopy of commassie stained bands