

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



Supernatant

Discard pellet



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



Discard supernatant

100,000 xg membranes

Saturated sucrose pad



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



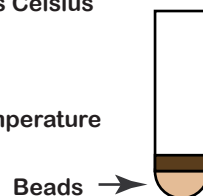
Remove precipitates by centrifugation at 10,000 xg for 30 min, collect supernatant



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



Elution immuno-complexes with specific peptide antigen at room temperature



Beads

Eluted complexes

- (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