



Title: RSV G Glycoprotein Crude Preparation:

No: RTLTP-PUR-1

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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•Lab coat	•Phosphate Buffered	•NaCl	•Pipettes
•Gloves	Saline	•dH ₂ O	•Pipetteman
•RSV-infected VeroE6 cells	•Wheat-germ lectin column	•Analytical balance	•Pipette Aid
•sonicator	•Tris HCL	•CHAPS	•Pipetteman tips
•GlcNAc	•pH Meter	•Ice bucket w/ ice	•Spectrophotometer
•Collection tubes	•1L Beaker	•Ultracentrifuge	•Dialysis Tubing
•Stir plate and stir bars	•Centrifuge	•Dialysis Clamps	

Buffers:

Binding Buffer:

20mM Tris, 0.5M NaCl pH 7.5 + 1% CHAPS

Wash Buffer:

20mM Tris, 0.5M NaCl pH 7.5 + 0.5% CHAPS

Elution Buffer:

0.5M GlcNAc/ 0.5% CHAPS in binding buffer

Dialysis Buffer:

Decided depending on desired application of product

Procedure:

1. Scrape RSV-infected VerE6 cells into PBS and pellet by centrifugation at 2500 rpm for 10 minutes.
2. Resuspend cells in binding buffer and stir the lysate for 30 minutes at room temperature.
3. Sonicate the lysate on ice 6 times for 30 seconds with a proper apparatus at 40% power.

4. Ultracentrifuge the lysate at 100k x g for 1 hour at 10°C.
5. Equilibrate a wheat germ lectin column with binding buffer per manufacturer's instructions.
6. Load the supernatant onto the column- the CHAPS soluble G protein will bind the wheat germ lectin.
7. Wash the column with wash buffer until the A_{280} of the effluent is at background (this should take ~20 mL).
8. Elute the column with elution buffer.
9. Remove CHAPS by dialysis vs. buffer of choice.