Title: *Purification of the RSV Fusion Glycoprotein G -Immunoaffinity Chromatography*

No: RTLP-GLP-LJ3b

Location:	Approval Date:	Supersedes Date:
Old CCRC Tripp Lab	5 October 2005	10 September 2004

- Lab coat
- Gloves
- anti-RSV G (131-2G)
- 0.2M NaHC03/ 0.5M NaCl pH 8.3
- Hi-Trap NHS activated 5mL column
- HCL
- 0.5M ethanolamine/0.5M NaCl pH 8.3
- 0.1M NaAcetate/0.5M NaCl pH 4
- PBS NaN3 0.05%
- Phosphate Buffered Saline (PBS)
- 0.1M Glycine
- 100mM EDTA
- Triton-x 100
- Sorvall Super T21 Centrifuge
- "Complete" protease inhibitor
- stir plate and stir bar
- 2M Tris pH 8.0
- RSV-infected Vero cells
- dH_2O
- Dialysis cassette
- Pipettes
- Pipetteman
- Pipette Aid
- Pipetteman tips
- -70°C Storage
- SonicDismembrator 500
- p960 pump (FPLC)

Procedure:

Prepare column:

1. 5ml of anti-RSV G (131-2G) 3.5 mg/ml was dialyzed overnight at 4C against 1L coupling buffer (0.2M NaHCO3 / 0.5M NaCl pH=8.3).
2. HiTrap NHS-activated 5ml column (Amersham) was washed with 30ml of ice cold 1mM HCL using a P960 pump (FPLC) at flow rate of 2ml/min.
3. 5ml of the antibody solution was manually injected on the column and incubated for 1hr at room temp.
4. 30ml of Buffer A (0.5M ethanolamine / 0.5M NaCl pH=8.3) was injected onto the column using a P960 pump (FPLC) at a flow rate of 2ml/min.
5. 30ml of Buffer B (0.1M NaAcetate / 0.5M NaCl pH=4) was injected onto the column using a P960 pump (FPLC) at a flow rate of 2ml/min.
6. 30ml of Buffer A (0.5M ethanolamine / 0.5M NaCl pH=8.3) was injected onto the column using a P960 pump (FPLC) at a flow rate of 2ml/min.
7. Incubate the column for 30min at room temp.
8. 30ml of Buffer B (0.1M NaAcetate / 0.5M NaCl pH=4) was injected onto the column using a P960 pump (FPLC) at a flow rate of 2ml/min.

9. 30ml of Buffer A (0.5M ethanolamine / 0.5M NaCl pH=8.3) was injected onto the column using a P960 pump (FPLC) at a flow rate of 2ml/min.
10. 30ml of Buffer B (0.1M NaAcetate / 0.5M NaCl pH=4) was injected onto the column using a P960 pump (FPLC) at a flow rate of 2ml/min.
11. 10ml of PBS pH=7.4 was injected onto the column using a P960 pump (FPLC) at a flow rate of 2ml/min.
12. Column is stored in PBS / NaN3 0.05% at 4C until use.

Prepare RSV Lysate:

1. RSV-infected Vero cells were collected by centrifugation at 7,000 rpm for 7min and washed 2X in ice cold PBS containing 1mM EDTA.
2. 2-3g of wet-weight cell pellet was resuspended in 40ml of ice cold PBS containing 5 "Complete" protease inhibitor tablets (Roche), and 1mM EDTA.
3. Cell slurry was started stirring on ice and 400ul of 10% TritonX-100 (Sigma) was added while stirring. Stirring continued for 30min.
4. Lysate was subjected to sonication at 80% power with a probe for 6 cycles of 2x30second blasts, with resting in ice bath for 5min between cycles.
5. Lysate was centrifuged at 12,500rpm for 15min.

Purification run:

1. Column is equilibrated with 25ml PBS / 0.05% TritonX-100 using a P960 pump (FPLC) at a flow rate of 2ml/min.
2. Load cell lysate onto the column using a P960 pump (FPLC) at a flow rate of 0.5 ml/min.
3. Wash column with 20ml PBS / 0.05% TritonX-100 using a P960 pump (FPLC) at a flow rate of 2ml/min.
4. Elute column with 20ml of 0.1M glycine / 0.05% TritonX-100 pH=2.2 using a P960 pump (FPLC) at a flow rate of 2ml/min, taking 4 x 10ml fractions.
5. Neutralize fractions with 1.5ml of 2M Tris pH=8.0
6. Fractions containing G protein are pooled and dialyzed against 3L of PBS / TritonX-100 0.05% at 4C overnight and concentrated to an appropriate volume using a Centricon 30 (Amicon) spin device.
7. Column is cleaned with 20ml of 0.1M glycine / 0.05% TritonX-100 pH=2.2 using a P960 pump (FPLC) at a flow rate of 2ml/min.
8. Column is re-equilibrated with 20ml PBS / 0.05% NaN3 using a P960 pump (FPLC) at a flow rate of 2ml/min, and stored at 4C.