



Title: *Immunostaining Plaque Assay for Respiratory
Syncytial Virus: From Stock Virus*

No: RTLP-PA-2

Location:
Old CCRC Tripp Lab

Approval Date:
12 July 2005

Supersedes Date:

Materials:

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•Lab coat	•24-well plates	•HBSS	•Pipettes
•Gloves	•D-MEM	•dH ₂ O	•Pipetteman
•Prepared Vero E6 cells	•37°C, 5% CO ₂ incubator	•Acteone	•Pipette Aid
• Virus Stock	•Centrifuge	•Methanol	•Pipetteman tips
•2% Methylcellulose	•PBS		•Bleach
•RSV mAb 131-2A	•Tween-20		•Microscope
•Goat-anti-mouse IgG whole molecule alkaline phosphatase	•Vector Black Kit		

Procedure:

**NOTE: 24 hours prior to plaque assay, 24-well plates of
Vero E6 cells must be seeded to obtain 80-90% confluency!**

1. Dilute virus 10 fold (10^{-1} to 10^{-6}) in D- MEM (Cellgro, Cat. # 10-013-CV) without serum.
2. Remove growth media and wash 24-well plate of Vero-E6 cells with 1X Hank's Balance Salt Solution (HBSS) (Hyclone, Cat. # SH30030-02).
3. Inoculate cells with 200 μ l/well of diluted virus (10^{-2} to 10^{-6}), in quadruplicate. Leave first column of cells as uninfected control (D-MEM only).
4. Adsorb virus for 1 hour at 37°C, 5 % CO₂.
5. After 1 hour, overlay cells with 1 ml/well of complete 2% Methylcellulose media (RTLP-PA-3)
6. Incubate cells for 6 days at 37°C, 5 % CO₂.

7. On Day 6 remove methylcellulose overlay (discard in 10% bleach solution).
8. Fix cells with ice-cold Acetone: Methanol (60:40) for 10 minutes and allow plates to air-dry.
9. Block cells in 5% nonfat dry milk (Carnation or equivalent) for 10 minutes at room temperature.
10. Remove 5% nonfat dry milk and add diluted antibody, RSV Monoclonal anti-F antibody, 131-2A (Chemicon, Cat. # MAB8599), diluted 1:800 in 5% nonfat dry milk. Incubate at 37°C for 2 hours.
11. Wash cells 2 X (5 min/wash) in PBS/0.5% Tween 20.
12. Add secondary antibody (Goat anti-mouse IgG whole molecule-Alkaline phosphatase conjugate (Sigma, Cat. # A3562) diluted 1:500 in 5% nonfat dry milk. Incubate at 37°C for 1 hour.
13. Wash cells 2 X (5 min/wash) in PBS/0.5% Tween 20.
14. Prepare Vector Black (Vector labs, Cat. # SK-5200) solution according the manufacturer's protocol.
15. Add substrate and develop reaction for at least 10 minutes (monitor by inverted light microscopy).
16. Stop reaction by adding DI H₂O to wells.
17. (Optional) Counterstain with Hemotoxylin (Fisher, Cat. # H-3401), and wash with H₂O.
18. Count stained plaques by inverted light microscopy at 4X magnification or with the Elispot reader.
19. For total plaques per ml: (# of plaques/well (total of all 4 replicates)/4 X 5 (dilution factor) = plaques per ml.