



Title: *Detection of Respiratory Syncytial Virus (RSV) by ELISA*

No: RTLP-GLP-EL-8

Location:
Old CCRC Tripp Lab

Approval Date:
13 December 2005

Supersedes Date:
10 September 2004

Materials:

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|----------------------|-------------------------|--------------------|---------------------------|
| •Lab coat | •Phosphate Buffered | •4°C cold storage | •Pipettes |
| •Gloves | Saline (PBS) | •Sigmafast pNpp | •Pipetteman |
| • RSV Antigen | •Tween-20 | Substrate (Sigma- | •Pipette Aid |
| • NaHCO ₃ | •2° Ab: goat anti-mouse | Aldrich N2770) | •Pipetteman tips |
| •Dry non-fat milk | IgG (H+L) whole | • negative control | •37°C, 5% CO ₂ |
| •1° Antibodies | molecule | = normal IgGκ, | incubator |
| | (Pierce cat # 31320) | (BD Pharmingen | |
| | | cat #553454) | |

Procedure:

1. Dilute your antigen (RSV-infected VeroE6 cell lysate or uninfected VeroE6 cell lysate) 1:1 PBSto a working concentration of 6-10μg/100μL.
2. Add 100 μL of antigen/PBS mixture to each test well and incubate at 37°C for 1 hour or overnight at 4°C.
3. Wash wells 3 X with PBS/0.5% Tween 20.
4. Block with 5% dry milk/PBS for 1 hour at 37°C or overnight at 4°C.
5. Add primary antibody (F protein positive control = clone 131-2A; G protein positive control = 130-2G; negative control = normal IgG1κ,(BD pharmingen cat #553454, test sera, or hybridoma supernatant) to wells at appropriate dilution (1:1000 for positive and negative; use undiluted (“Neat”) for supernatants or unknowns)
6. Incubate at 37°C for 1 hour or overnight at 4°C.
7. Wash wells 3 X with PBS/0.5% Tween 20.

8. Add secondary antibody (Goat Anti-mouse IgG (H+L) whole molecule-alkaline phosphatase conjugated) diluted 1:1000 to wells (100 µl/well).
9. Incubate at 37°C for 1 hour or overnight at 4°C.
10. Wash 3 X with PBS/0.5% Tween 20
11. Make up the pNpp (Sigmafast) substrate Sigma Aldrich N2770 accordingly. Need 20 ml/plate.
12. Add 200 µl of substrate/well and incubate for 10-15 minutes based on reaction and background.
13. Measure absorbance at OD 405/495.