Product Manual

RSV Fusion Protein ELISA Kit

Catalog Numbers

VPK-5170 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Respiratory syncytial virus (RSV) infection is a significant cause of hospitalization of children and one of the leading causes of death of infants less than 1 year of age worldwide. RSV is a member of the *Pneumoviridae* genus and contains a single-stranded non-segmented negative-sense RNA genome approximately 15,200 nt in length. There are three surface proteins that coat the virion: small hydrophobic (SH), attachment (G), and fusion (F). G and F proteins are the major antigenic proteins. RSV is categorized into subgroups A and B based on the sequence of the G protein.

RSV-F protein is a trimeric transmembrane glycoprotein that mediates binding of RSV to cellular receptors and induces pH-independent fusion between the viral envelope and the cellular plasma membrane. The F glycoprotein is highly conserved among RSV isolates from both A and B subgroups, with over 90% amino acid sequence identities.

Cell Biolabs' RSV Fusion Protein ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the RSV fusion protein. The ELISA antibodies recognize the fusion protein from both RSV type A and type B. The kit has a detection sensitivity limit of 156 pg /mL RSV-F. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and RSV lysate samples.

Assay Principle

An anti-RSV fusion protein monoclonal coating antibody is adsorbed onto a microtiter plate. RSV fusion protein present in the sample or standard binds to the antibodies adsorbed on the plate; an FITC-conjugated anti-RSV fusion protein monoclonal antibody is added and binds to the RSV fusion protein captured by the first antibody. Following incubation and wash steps, a HRP-conjugated mouse anti-FITC antibody is added and binds to the FITC conjugated anti- RSV fusion protein monoclonal antibody. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of RSV fusion protein present in the sample. The reaction is terminated by addition of Stop Solution and absorbance is measured at 450 nm. A standard curve is prepared from RSV fusion protein standard and sample RSV fusion protein concentration is then determined.

Related Products

1. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV-1 p24 ELISA)
2. VPK-150: QuickTiter™ Hepatitis B Core Antigen (HBcAg) ELISA Kit
3. VPK-151: QuickTiter™ Hepatitis C Core Antigen (HCcAg) ELISA Kit
4. VPK-156: QuickTiter™ MuLV Core Antigen (MuLV p30) ELISA Kit
5. VPK-5169: Adenovirus Hexon ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-RSV-F Antibody Coated Plate (Part No. 51701B): One strip well 96-well plate.

2. FITC-Conjugated Anti-RSV-F Monoclonal Antibody (Part No. 51702C): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Viral Lysis Buffer (Part No. 51693B): One 15 mL bottle containing 200 mM Tris, pH 7.5, 1500 mM NaCl, 10% Triton X-100, 1% SDS.
6. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
7. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
8. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. RSV Fusion Protein Standard (Part No. 51703D): One 100 µL vial of 1 µg/mL recombinant human RSV fusion protein in PBS containing BSA.

Materials Not Supplied

1. RSV Sample: purified virus or unpurified viral supernatant
2. Microcentrifuge
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store the RSV Fusion Protein Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Safety Considerations

Remember that your RSV samples contain infectious viruses before inactivation; you must follow the recommended NIH guidelines for all materials containing infectious organisms.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-RSV-F Monoclonal Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of RSV Fusion Protein Standard in the concentration range of 10 ng/mL – 0.156 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL RSV Fusion Protein Standard (µL)	Assay Diluent (µL)	RSV F (ng/mL)
1	10	990	10
2	500 of Tube #1	500	5
3	500 of Tube #2	500	2.5
4	500 of Tube #3	500	1.25
5	500 of Tube #4	500	0.625
6	500 of Tube #5	500	0.313
7	500 of Tube #6	500	0.156
8	0	500	0

Table 1. Preparation of RSV Fusion Protein Standard

2. Transfer 225 µL of each dilution to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer. Perform the assay as described in Assay Protocol.

RSV Sample Inactivation and Lysis

1. (Optional) Dilute RSV samples in culture medium. Include culture medium as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of 10X Lysis Buffer, vortex well. Inactivate RSV sample at 56ºC for 30 min.
3. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as RSV lysate.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each RSV lysate sample, RSV fusion protein standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of RSV lysate or RSV fusion protein standard to Anti-RSV-F Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37ºC for 2 hours.
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted FITC-Conjugated Anti-RSV-F Monoclonal Antibody to each well.
7. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

8. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 5 above.
9. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
10. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
11. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 5 above. Proceed immediately to the next step.
12. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
13. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
14. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical RSV Fusion Protein ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

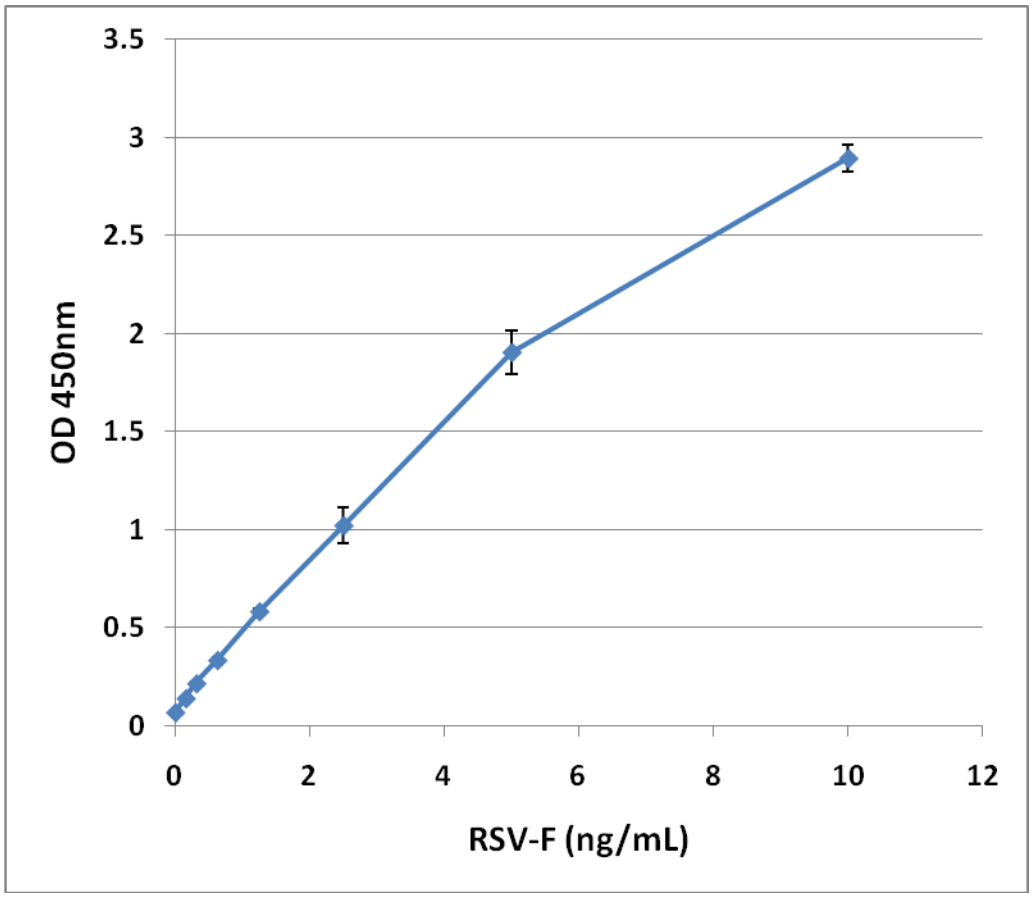

Figure 1: RSV Fusion Protein ELISA Standard Curve

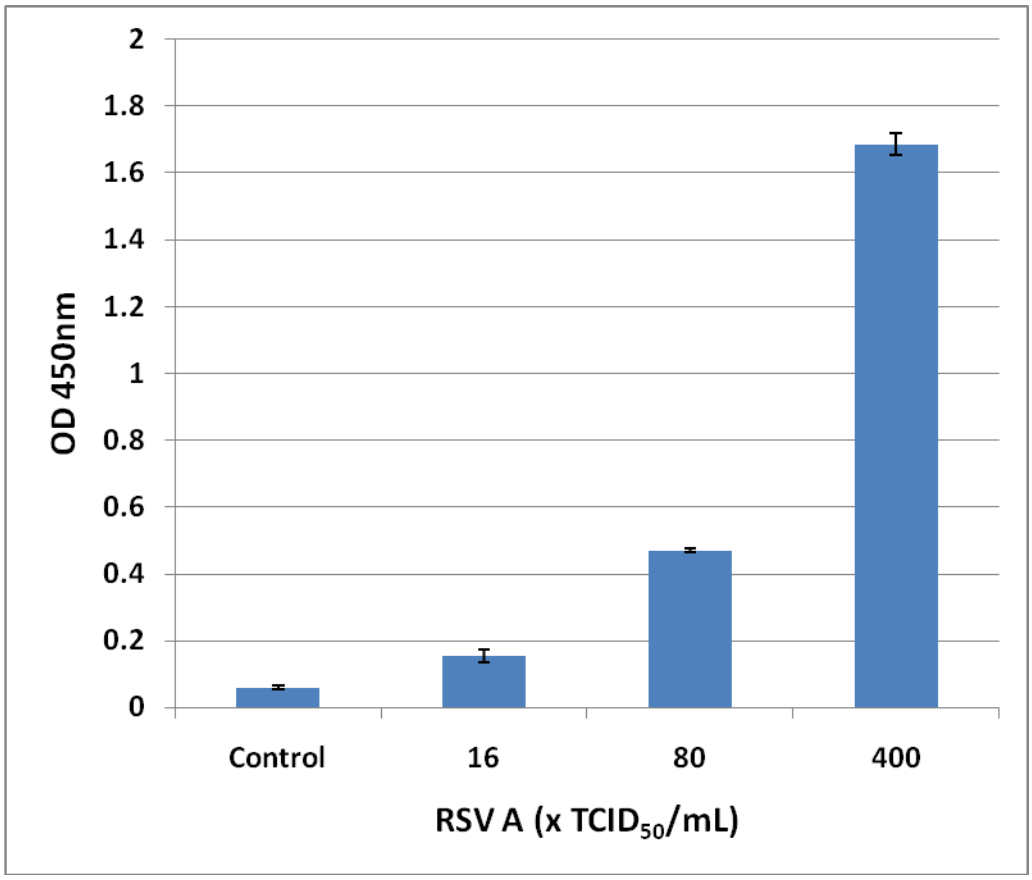

Figure 2: RSV fusion protein in RSV Culture Fluid. RSV A culture fluid (1.05 x 10^6 $TCID_{50}$/mL) was first diluted 1000-fold with culture medium, then heat inactivated and lysed in Viral Lysis Buffer. RSV lysate was subjected to RSV Fusion Protein ELISA Kit according to Assay Protocol.

References

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3. Teng, M. N., Whitehead, S. S. & Collins, P. L. (2001) Contribution of the respiratory syncytial virus G glycoprotein and its secreted and membrane-bound forms to virus replication in vitro and in vivo. *Virology* **289**, 283–296.
4. McLellan, J. S. et al. (2013) Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. *Science* **340**, 1113–1117.
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6. Gonzalez-Reyes, L. et al. (2001) Cleavage of the human respiratory syncytial virus fusion protein at two distinct sites is required for activation of membrane fusion. *Proc. Natl. Acad. Sci. USA* **98**, 9859–9864.

Warranty

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