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Product Manual

# RSV Nucleoprotein ELISA Kit

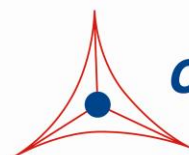
Catalog Numbers

VPK-5171

96 assays

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **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 nucleoprotein (N) is essential for virus assembly and replication. It is essential for virus encapsidation by coating the entire viral RNA genome to form the ribonucleoprotein. The RSV nucleoprotein is highly conserved among RSV isolates from both A and B subgroups, with over 95% amino acid sequence identities.

Cell Biolabs' RSV Nucleoprotein ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the RSV nucleoprotein. The ELISA antibodies recognize the nucleoprotein of both RSV type A and type B. The kit has a detection sensitivity limit of 313 pg/mL RSV nucleoprotein. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and RSV lysate samples.

## **Assay Principle**

An anti-RSV nucleoprotein monoclonal coating antibody is adsorbed onto a microtiter plate. RSV nucleoprotein present in the sample or standard binds to the antibodies adsorbed on the plate; an FITC-conjugated anti-RSV nucleoprotein polyclonal antibody is added and binds to the RSV nucleoprotein 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 nucleoprotein polyclonal antibody. Unbound HRP-conjugated mouse anti-FITC antibody is removed during a wash step, and a substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of RSV nucleoprotein 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 recombinant RSV nucleoprotein standard and sample RSV nucleoprotein 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
6. VPK-5170: RSV Fusion Protein ELISA Kit
7. VPK-5174: Influenza A Nucleoprotein ELISA Kit
8. VPK-5175: Influenza B Nucleoprotein ELISA Kit

## **Kit Components**

### **Box 1 (shipped at room temperature)**

1. Anti-RSV Nucleoprotein Antibody Coated Plate (Part No. 51711B): One strip well 96-well plate.
2. FITC-Conjugated Anti-RSV Nucleoprotein Polyclonal Antibody (Part No. 51712C): One 20  $\mu$ L vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20  $\mu$ L 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. Recombinant RSV Nucleoprotein Standard (Part No. 51713D): One 100  $\mu$ L vial of 2  $\mu$ g/mL recombinant human RSV nucleoprotein (Met1-Leu391) in PBS containing BSA.

## **Materials Not Supplied**

1. RSV Sample: purified virus or unpurified viral supernatant
2. Microcentrifuge
3. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
4. 50  $\mu$ L to 300  $\mu$ L 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 Nucleoprotein Standard at  $-20^{\circ}\text{C}$  and avoid freeze/thaw. Store all other components at  $4^{\circ}\text{C}$ .

## **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 Nucleoprotein Polyclonal 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 Nucleoprotein Standard in the concentration range of 20 ng/mL – 0.313 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

| <b>Standard Tubes</b> | <b>2 µg/mL RSV Nucleoprotein Standard (µL)</b> | <b>Assay Diluent (µL)</b> | <b>RSV Nucleoprotein (ng/mL)</b> |
|-----------------------|--|---------------------------|----------------------------------|
| 1                     | 10   | 990                       | 20                               |
| 2                     | 500 of Tube #1                                 | 500                       | 10                               |
| 3                     | 500 of Tube #2                                 | 500                       | 5                                |
| 4                     | 500 of Tube #3                                 | 500                       | 2.5                              |
| 5                     | 500 of Tube #4                                 | 500                       | 1.25                             |
| 6                     | 500 of Tube #5                                 | 500                       | 0.625                            |
| 7                     | 500 of Tube #6                                 | 500                       | 0.313                            |
| 8                     | 0  | 500                       | 0                                |

**Table 1. Preparation of RSV Nucleoprotein 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 56°C for 30 min.
3. Centrifuge at 12,000 x g for 5 minutes at 4°C. Collect the supernatant as RSV lysate.

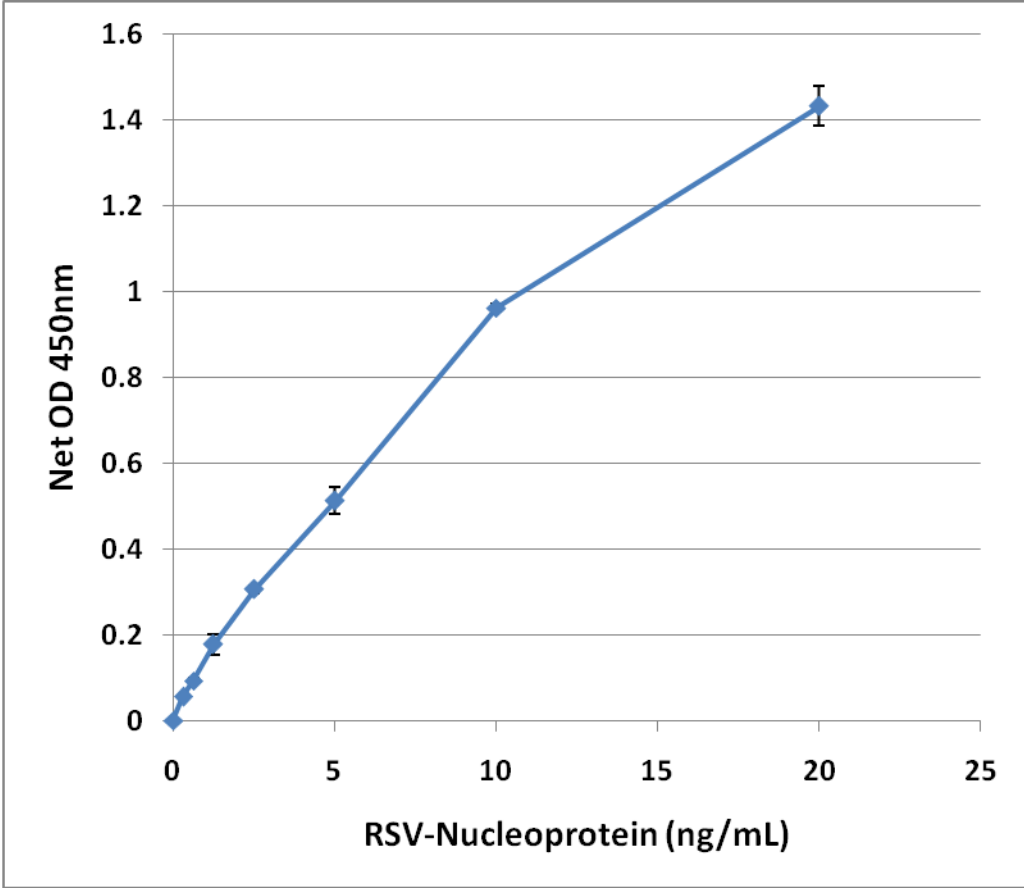
### **Assay Protocol**

1. Prepare and mix all reagents thoroughly before use.
2. Each RSV lysate sample, RSV nucleoprotein standard, blank, and control medium should be assayed in duplicate.
3. Add 100 µL of RSV lysate or RSV nucleoprotein standard to Anti-RSV Nucleoprotein Antibody Coated Plate.
4. Cover with a Plate Cover and incubate at 37°C for 2 hours.

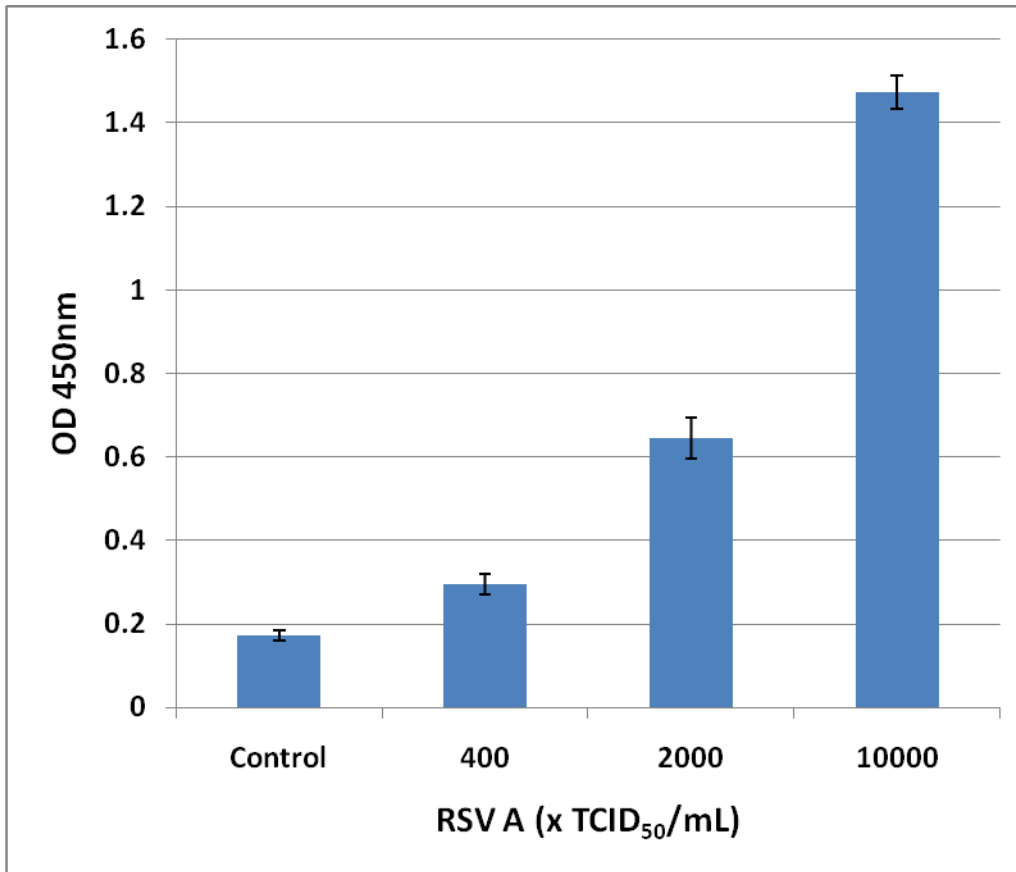
5. Remove plate cover and empty wells. Wash microwell strips 5 times with 250  $\mu$ L 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100  $\mu$ L of the diluted FITC-Conjugated Anti-RSV Nucleoprotein polyclonal 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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 Nucleoprotein ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: RSV Nucleoprotein ELISA Standard Curve**



**Figure 2: RSV Nucleoprotein in RSV Culture Fluid.** RSV A culture fluid ( $1.05 \times 10^6$  TCID<sub>50</sub>/mL) was first diluted 1000-fold with culture medium, then heat inactivated and lysed in Viral Lysis Buffer. RSV lysate was tested in the RSV Nucleoprotein ELISA Kit according to the Assay Protocol.

## References

1. Afonso, C. L. et al. (2016) Taxonomy of the order Mononegavirales. *Arch. Virol.* **161**, 2351–2360.
2. Shi, T. et al. (2017) Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* **390**, 946–958.
3. Bhella D., Ralph A., Murphy L. B., Yeo R. P. (2002) Significant differences in nucleocapsid morphology within the Paramyxoviridae. *J Gen Virol* **83**, 1831–1839.
4. Tawar R. G., Duquerroy S., Vonnhein C., Varela P. F., Damier-Piolle L., Castagné N., MacLellan K., Bedouelle H., Bricogne G.. (2009) Crystal structure of a nucleocapsid-like nucleoprotein-RNA complex of respiratory syncytial virus. *Science* **326**, 1279–1283.

## Warranty

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