



A178-EN.02

HRSV Pre-Fusion glycoprotein F0(site V) Specific ELISA Kit (For Vaccine Development)

Pack Size: 96 tests

Catalog Number: RAS-A178

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

This kit is developed for specific quantitative detection of HRSV Pre-Fusion glycoprotein F0(site V) in samples.

This kit can specifically identify HRSV (A) and HRSV (B) Pre-Fusion glycoprotein F0 by testing.

PRINCIPLE OF THE ASSAY

Respiratory syncytial virus (RSV) is a highly contagious virus causing severe infection in infants and the elderly. Various approaches are being used to develop an effective RSV vaccine. The RSV fusion (F) subunit, particularly the cleaved trimeric pre-fusion F, is one of the most promising vaccine candidates under development. A rapid and effective assay kit detecting the levels of HRSV Pre-Fusion glycoprotein F0 is urgently needed to accelerate the development of RSV vaccines.

This assay kit is used to measure the levels of HRSV Pre-Fusion glycoprotein F0(site V) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-RSV-Pre-Fusion glycoprotein F0 (site V) Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the HRP-Anti-Fusion glycoprotein F0 (RSV) Antibody to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of Pre-Fusion glycoprotein F0(site V) present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of HRSV Pre-Fusion glycoprotein F0(site V) bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

| Catalog | Components | Size (96 tests) | Format | Storage | |
|------------|---|--------------------|--------|--------------------|--------------------|
| | | | | Unopened | Opened |
| RAS178-C01 | Pre-coated Anti-RSV-Pre-Fusion glycoprotein F0 (site V) Antibody Microplate | 1 plate | Solid | 2-8°C | 2-8°C |
| RAS178-C02 | Pre-Fusion glycoprotein F0 (RSV) Standard | 20 µg | Powder | 2-8°C | -70°C |
| RAS178-C03 | HRP-Anti-Fusion glycoprotein F0 (RSV) Antibody | 20 µg | Powder | 2-8°C, avoid light | -70°C, avoid light |

| | | | | | |
|------------|--------------------|-------|--------|--------------------|--------------------|
| RAS178-C04 | 10×Washing Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| RAS178-C05 | 2×Dilution Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| RAS178-C06 | Substrate Solution | 12 mL | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| RAS178-C07 | Stop Solution | 7 mL | Liquid | 2-8°C | 2-8°C |

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Centrifuge;

37°C Incubator;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10× Washing Buffer;

STORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.
2. The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in a 37 °C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.
2. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2 and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70°C. It is recommended not to freeze-thaw

more than 1 times, the packing specification shall not be less than 5 µg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

| ID | Components | Size | Stock Solution Con. | Reconstitution Buffer and Vol. |
|------------|--|-------|---------------------|--------------------------------|
| RAS178-C02 | Pre-Fusion glycoprotein F0 (RSV) Standard | 20 µg | 200 µg/mL | 100 µL |
| RAS178-C03 | HRP-Anti-Fusion glycoprotein F0 (RSV) Antibody | 20 µg | 100 µg/mL | 200 µL |

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

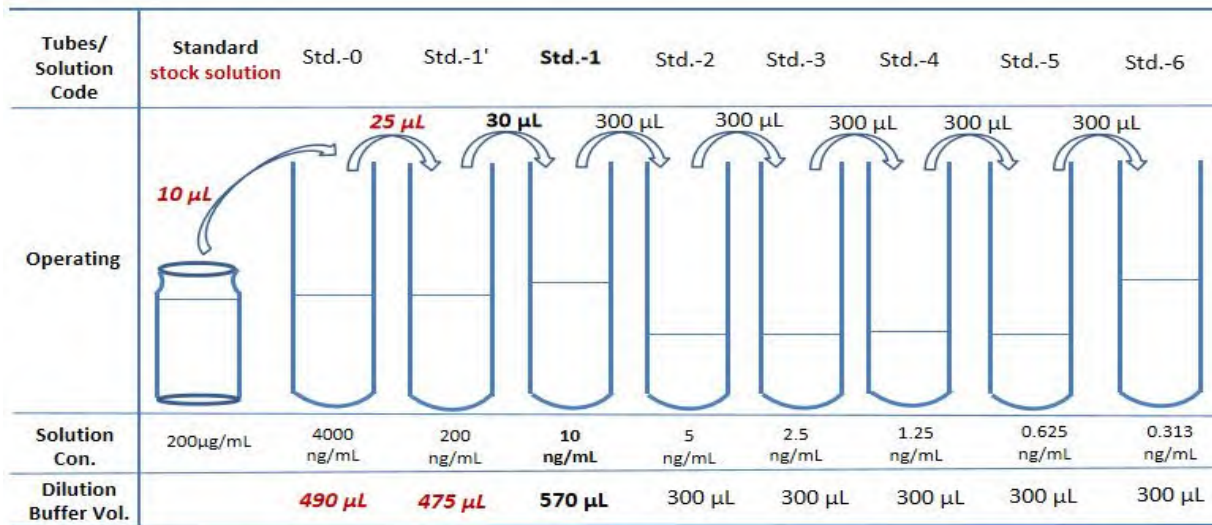
1.3 Preparation of HRP-Anti-Fusion glycoprotein F0 (RSV) Antibody working fluid:

Dilute HRP-Anti-Fusion glycoprotein F0 (RSV) Antibody to 0.05 µg/mL with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard curve

Make serial dilutions of the Pre-Fusion glycoprotein F0 (RSV) as a Standard curve with Dilution Buffer as recommended in Figure 1.

FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Pre-F0 (RSV)



3. Add Samples

Add 100 μ L serially diluted Pre-Fusion glycoprotein F0 (RSV) Standard curve and samples to each well. For blank Control wells, please add 100 μ L 1 \times Dilution Buffer. Seal the plate with microplate sealing film and incubate at 37 $^{\circ}$ C for 1 hour.

4. Washing

Remove the remaining solution by aspiration, add 300 μ L of 1 \times Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1 \times Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

5. Add HRP-Anti-Fusion glycoprotein F0 (RSV) Antibody

For all wells, add 100 μ L **HRP-Anti-Fusion glycoprotein F0 (RSV) Antibody (dilute to 0.05 μ g/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37 $^{\circ}$ C for 1 hour.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 μ L **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37 $^{\circ}$ C for 20 min, avoid light.

8. Termination

Add 50 μ L **Stop Solution** to each well, and tap the plate gently for 5 min to allow thorough mixing.

Note: the color in the wells should change from blue to yellow.

9. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer.

Note: To reduce the background noise, subtract the value read at OD_{450 nm} with the value read at OD_{630 nm}.

CALCULATION OF RESULTS

1. Normal range of Standard curve: $R^2 \geq 0.9900$, detection range: 0.313-10 ng/mL.
2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with

dilution buffer and assay repeated.

3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20 °C -25 °C) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C.

TYPICAL DATA

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

| Pre-Fusion glycoprotein F0 (RSV) Standard(ng/mL) | OD450-630nm | OD450-630nm-Blank |
|--|-------------|-------------------|
| 10 | 1.998 | 1.988 |
| 5 | 0.969 | 0.958 |
| 2.5 | 0.457 | 0.446 |
| 1.25 | 0.215 | 0.204 |
| 0.625 | 0.112 | 0.102 |
| 0.313 | 0.059 | 0.049 |
| Blank | 0.011 | 0.000 |

