RA202-EN.01

# HRSV Pre-Fusion glycoprotein F0 (site V) Specific ELISA Kit

**Pack Size: 96 tests**

**Catalog Number：**RAS-A202

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

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

## INTENDED USE

The kit has been tested to specifically identify HRSV (A) and HRSV (B) Pre-Fusion glycoprotein. It was developed for the specific quantitative detection of HRSV Pre-Fusion glycoprotein F0 (site V) in samples. It is intended for research use only (RUO).

## 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.

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-Pre-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 HRSV 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 |
| RAS202-C01 | Pre-coated Anti-RSV-Pre-Fusion glycoprotein F0 (site V) Antibody Microplate | 1 plate | Solid | 2-8°C | 2-8°C |
| RAS202-C02 | Pre-Fusion glycoprotein F0 (RSV) Standard | 15 μg | Powder | 2-8°C | -70°C |
| RAS202-C03 | HRP-Anti-Pre-Fusion glycoprotein F0 (RSV) Antibody | 20 μg | Powder | 2-8°C, avoid light | -70°C, avoid light |
| RAS202-C04 | 10×Washing Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |



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| | | | | | |
|---|---|---|---|---|---|
| RAS202-C05 | 2×Dilution Buffer | 50 mL | Liquid | 2-8℃ | 2-8℃ |
| RAS202-C06 | Substrate Solution | 12 mL | Liquid | 2-8℃, avoid light | 2-8℃, avoid light |
| RAS202-C07 | Stop Solution | 7 mL | Liquid | 2-8℃ | 2-8℃ |

## REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

Centrifuge;

37ºC 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℃-8℃ upon receiving.

2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

## REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in a 37 ºC 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℃. It is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5 µg.



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**TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS**

| ID | Components | Size | Stock Solution Con. | Reconstitution Buffer and |
|---|---|---|---|---|
| RAS202-C02 | Pre-Fusion glycoprotein F0 (RSV) Standard | 15 μg | 150 μg/mL | 100 μL water |
| RAS202-C03 | HRP-Anti-Pre-Fusion glycoprotein F0 (RSV) Antibody | 20 μg | 200 μg/mL | 100 μL water |

## 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.

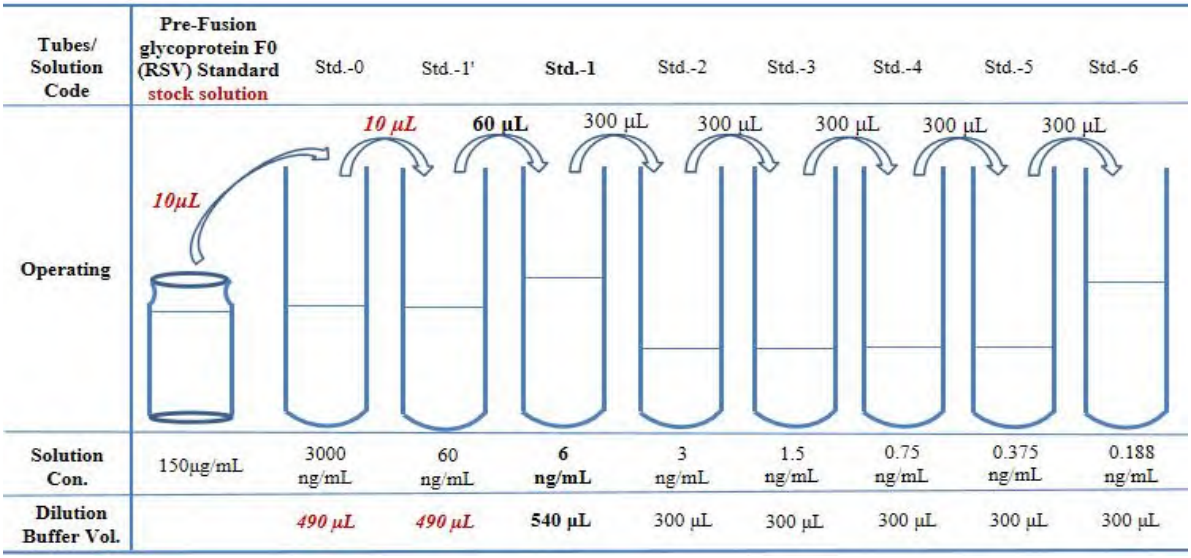
1.3 Preparation of HRP-Anti-Pre-Fusion glycoprotein F0 (RSV) Antibody working fluid:

Dilute HRP-Anti-Pre-Fusion glycoprotein F0 (RSV) Antibody to 0.2 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-Fusion glycoprotein F0 (RSV)**



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### 3. Add Samples

Add 100μL serially diluted **Pre-Fusion glycoprotein F0 (RSV)** Standard curve and samples to each well. For blank Control wells, please add 100 μL 1×Dilution Buffer. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 4. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, soak for 30s, remove any remaining 1×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-Pre-Fusion glycoprotein F0 (RSV) Antibody

For all wells, add 100 μL **HRP-Anti-Pre-Fusion glycoprotein F0 (RSV) Antibody (dilute to 0.2 μg/mL)** working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

### 6. Washing

Repeat step 4.

### 7. Substrate Reaction

Add 100 μL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

### 8. Termination

Add 50 μL **Stop Solution** to each well and tap the plate gently 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 within 5 minutes.

***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: 0.188-6 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.



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3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted from 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.

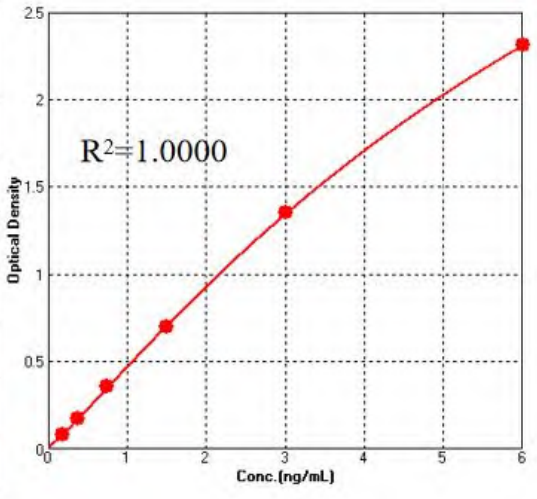
## PRECAUTIONS

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℃-25℃) 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.

| Standard (ng/mL) | O.D.-1 | O.D.-2 | Average | Corrected |
|---|---|---|---|---|
| 6 | 2.320 | 2.364 | 2.342 | 2.311 |
| 3 | 1.389 | 1.375 | 1.382 | 1.351 |
| 1.5 | 0.741 | 0.724 | 0.733 | 0.702 |
| 0.75 | 0.388 | 0.393 | 0.391 | 0.360 |
| 0.375 | 0.208 | 0.207 | 0.208 | 0.177 |
| 0.188 | 0.115 | 0.117 | 0.116 | 0.085 |
| 0 | 0.035 | 0.027 | 0.031 | / |




## PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision



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Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 10 | 10 | 10 | 3 | 3 | 3 |
| Mean (ng/mL) | 4.394 | 0.848 | 0.435 | 4.623 | 0.894 | 0.463 |
| SD | 0.255 | 0.034 | 0.024 | 0.213 | 0.046 | 0.040 |
| CV (%) | 5.8 | 4.0 | 5.5 | 4.6 | 5.2 | 8.7 |

***Note**: The example data is for reference only.*

## RECOVERY

Three samples with different concentrations were tested to calculate the recovery rate.

| Sample(n=5) | Average Recovery % | Range % |
|---|---|---|
| High | 98.0 | 90.5-104.7 |
| Middle | 97.9 | 91.5-105.4 |
| Low | 99.9 | 91.8-107.4 |

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture medium (DMEM) | Cell culture medium (1640) |
|---|---|---|---|
| 1:2 | Average Recovery (%) | 90.5 | 94.5 |
| | Range (%) | 84.9-97.0 | 91.2-97.9 |
| 1:4 | Average Recovery (%) | 94.3 | 97.5 |
| | Range (%) | 86.9-101.2 | 95.4-99.2 |
| 1:8 | Average Recovery (%) | 103.6 | 106.9 |
| | Range (%) | 101.2-106.8 | 103.0-109.3 |



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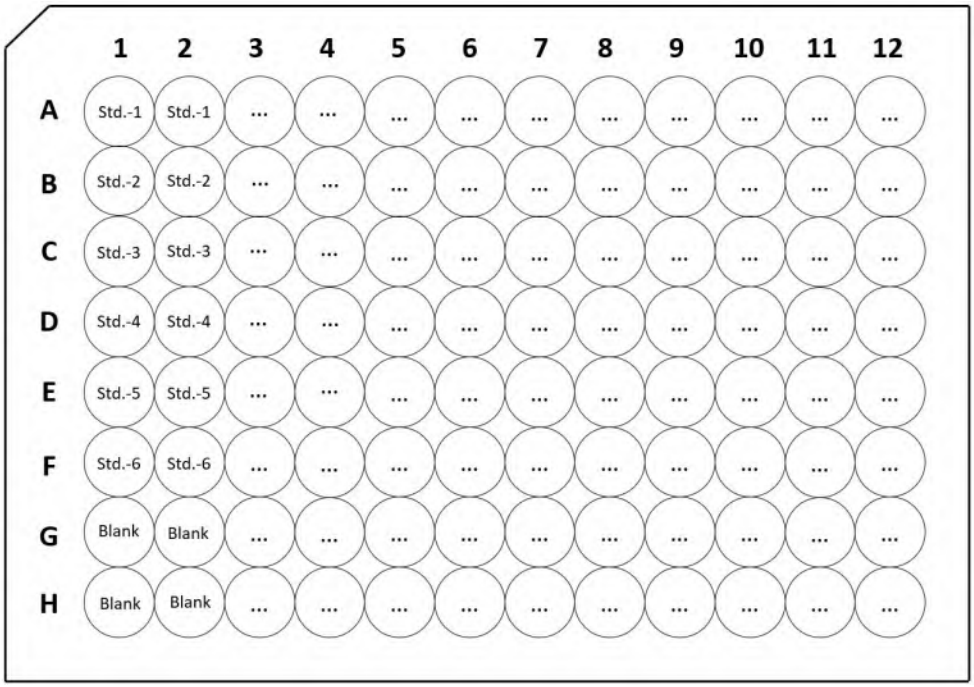
| | | | |
|---|---|---|---|
| 1:16 | Average Recovery (%) | 113.4 | 117.7 |
| | Range (%) | 105.9-119.9 | 116.6-119.6 |

***Note**: The example data is for reference only.*

## PLATE LAYOUT




***Note:** Blank is a Blank Dilution Buffer hole.*

## TROUBLESHOOTING GUIDE

| Problem | Cause | Solution |
|---|---|---|
| **Poor standard curve** | * Inaccurate pipetting | * Check pipettes |
| **Large CV** | * Inaccurate pipetting<br>* Air bubbles in wells | * Check pipettes<br>* Remove bubbles in wells |
| **High background** | * Plate is insufficiently washed<br>* Contaminated wash buffer | * Review the manual for proper wash.<br>* Make fresh wash buffer |
| **Very low readings across the plate** | * Incorrect wavelengths<br>* Insufficient development time | * Check filters/reader<br>* Increase development time |



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| | | |
|---|---|---|
| **Samples are reading too high, but standard curve looks fine** | * Samples contain cytokine levels above assay range | * Dilute samples and run again |
| **Drift** | * Interrupted assay set-up<br>* Reagents not at room temperature | * Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay<br>* Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts |



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