Version 30-04/24

User's Manual

RSV Pre-F ELISA Kit

REF **DEIA-NS2402-RSV1**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit uses a double-antibody sandwich enzyme-linked immunoassay (ELISA) to detect RSV Pre-F, which can detect and quantitatively analyze RSV Pre-F content with high sensitivity and specificity.

General Description

Respiratory syncytial virus (RSV) is one of the important pathogens causing acute lower respiratory tract infections in infants, young children, the elderly and people with low immunity. The fusion protein (F) on the surface of the virus is a highly conserved and highly antigenic type I mucosal glycoprotein, which is divided into two conformations: pre-fusion (Pre-F) and post-fusion (Post-F). Pre-F protein contains 6 antigenic epitopes, among which the antigenic epitopes only exist in Pre-F protein, and the antibodies induced against this epitope have the highest neutralizing activity.

Principles of Testing

This kit uses a double-antibody sandwich ELISA method. It is pre-coated with RSV Pre-F capture antibody. After adding the sample, the sample is captured to form an antibody-antigen complex, and then the HRP-labeled RSV Pre-F detection antibody is added to form an antibody-antigen-antibody "sandwich" complex. Finally, TMB is added for color development. After the reaction is terminated, the absorbance value (OD value) is read at a wavelength of 450nm/630nm. The content of RSV Pre-F in the sample is positively correlated with the OD value.

Reagents And Materials Provided

1. RSV Pre-F Microplate, 96T/kit
2. RSV Pre-F Detection Antibody, 150 µL
3. RSV Pre-F Standard, 5ug/mL, 150uL
4. 20× Wash Buffer, 30mL
5. Dilution Buffer 1, 30mL
6. Dilution Buffer 2, 15mL
7. Color Reagent A, 6mL
8. Color Reagent B, 6mL
9. Stop Reagent, 6mL
10. Sealing Film, 2

Storage

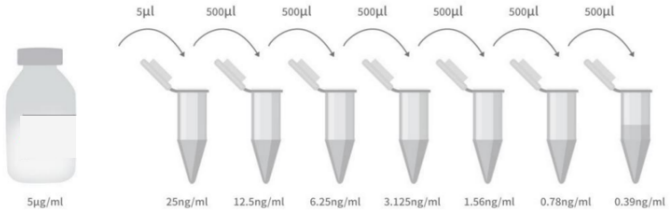
The kit is unopened and stored in a dry place at 2-8°C away from light. It is valid for 12 months. Please use the kit as soon as possible after opening it.

Reagent Preparation

All reagent components and samples to be tested need to return to room temperature before use.

1. Preparation of 1×Wash Buffer: Equilibrate 20×Wash Buffer to room temperature until the crystals are completely dissolved. After mixing, dilute 20 times with deionized water or ultrapure water. The dosage of diluted 1×Wash Buffer per well is 3ml. , stable for 1 week at 2-8°C
2. Dilution of RSV Pre-F Detection Antibody: Mix well before use, and then use Dilution Buffer 2 to dilute PPase Detection Antibody at a dilution ratio of 1:100.
3. Preparation of substrate solution: Mix Color Reagent A and Color Reagent B at 1:1 and mix immediately. The dosage per well is 100ul. If the mixed substrate solution turns blue, do not use it.
4. Dilution of RSV Pre-F Standard: Take 5ul of RSV Pre-F Standard (5pg/ml), add 995ul Dilution Buffer 1 to dilute to 25ng/ml concentration, take 500µl of 25ng/ml concentration sample into a new EP tube, add 500µl Dilution Buffer 1 was diluted 2-fold to 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0ng/ml respectively.

Note: To ensure the validity of experimental results, please use newly prepared standard solution for each experiment.



Assay Procedure

All operations are performed at room temperature. It is recommended that the standards and samples to be tested be measured in duplicate.

1. Sample incubation: Add the diluted RSV Pre-F Standard and the sample to be tested to the enzyme plate respectively, 100µl/well, mix well, cover with sealing film, and incubate at 37C for 1 hour; *When the content of RSV Pre-F in the sample is uncertain, it is recommended to test the sample at multiple dilutions to avoid being unable to obtain valid readings due to too high content.
2. Wash the plate: discard the liquid in the well, wash the plate with 1×Wash Buffer, 300µl/well, wash the plate 5 times, and pat the remaining liquid as dry as possible for the last time;
3. Detection antibody incubation: Add the diluted RSV Pre-F Detection Antibody to the enzyme plate, 100µl/well, mix well, cover with sealing film, and incubate at 37°C for 1 hour;
4. Wash the plate: Repeat step 2:
5. Color development: Add the prepared substrate solution to the enzyme plate, 100µl/well, mix well, cover with sealing film, and incubate at 37°C in the dark for 20 minutes;
6. Termination: Add Stop Reagent to the microplate, 50µl/well, shake the microplate gently until the color develops evenly, and ensure that the color development time of each well is the same;
7. Reading value: Place the enzyme plate into the microplate reader. It is recommended to read the absorbance using dual-wavelength testing (test wavelength, reference wavelength 630nm), and read the

value within 20 minutes.

Calculation

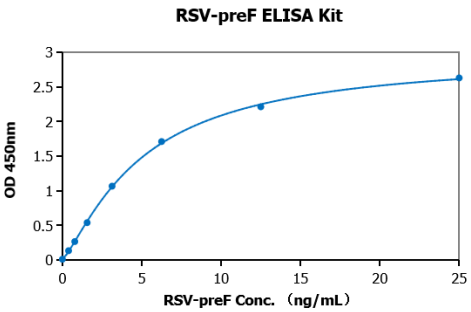
If the OD value of the sample to be tested exceeds the OD value at the highest point of the standard curve, the sample needs to be diluted and remeasured.

Draw a standard curve with the concentration of the standard substance as the abscissa and the absorbance value of the standard substance as the ordinate. It is recommended to use ELISACalc.exe regression and fitting calculation software to perform four-parameter fitting to calculate the RSV Pre-F concentration in the sample.

Typical Standard Curve

The following standard curve chart is for reference only. The standard curve drawn for the same experimental standard should be used to calculate the sample content.

ng/mL	OD450
25	2.628
12.5	2.211
6.25	1.709
3.13	1.065
1.56	0.538
0.78	0.265
0.39	0.133
0	0.016
R^2	0.9996



Precision

CV of the ELISA kit is less than 15%.

Detection Range

0.78-25ng/mL

Sensitivity

0.78ng/mL

Recovery

80%-120%