PRODUCT INFORMATION & MANUAL

SARS-CoV-2 Spike RBD Antibody (Mouse IgG) - Delta Variant, B.1.617.2, India ELISA Kit (Colorimetric)

NBP3-21490

Sample Insert for Reference Only

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Intended Use

The kit applies for detecting the level of anti-SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India antibody in serum or plasma of vaccinated.

PRINCIPLE OF THE ASSAY

The principle of the kit is indirect ELISA. SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India Recombinant Protein has been pre-coated onto well plate strips. The samples or control antibody are added to the well, after incubation the wells are washed and a horseradish peroxidase conjugated HRP-Rabbit anti-Mouse IgG is added, producing a complex "antigen-antibody-labeled antibody". Following a wash, the TMB substrate solution is loaded and color develops in proportion to the amount of antibodies. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm.

KIT CONTENTS AND STORAGE

The kit, if unopened, is stable at 2-8℃ for 6 months upon receipt.

Components	Amt	Preparation instructions	storage
Microplate: Pre-coated with SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India Recombinant Protein	1 plate (96 tests)	Take the microplate strips as needed, and put the unused strips back to the vacuum bag. It is best to vacuumize them	Vacuum storage can store at 2-8℃ until expiration date and opened package store at 2-8℃ for 1month.
HRP-Rabbit anti-Mouse IgG	1 vial	Dilute at 1:100 with 1×dilution buffer for 10 minutes before use. Dilute fresh as needed.	primary liquid are stable at 2 - 8℃until expiration date .To be reconstituted, The working fluid is used within the working day and discard. So dilute fresh as needed.
Anti- SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India (Control)	1 vial	Gradient diluted at 1:80000-1:600000 with 1×dilution buffer for 10 minutes before use (As a qualitative control, diluted at1:160000, OD_{450nm}-Blank>1.0). Dilute fresh as needed.	
20 × Dilution Buffer	1 bottle	Prepare 1×Dilution Buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer. **Dilute fresh as needed.**	
20 × Wash Buffer	1 bottle	Prepare 1×Wash Buffer by adding 15 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 300 mL of Wash Buffer. **Dilute fresh as needed.**	
Color Reagent A	1 bottle	Color Reagents A and B should be mixed together in equal volumes within 10 minutes before use. Take care not to contaminate the Color Reagent. If the mixed color reagent is blue, DO NOT USE	
Color Reagent B	1 bottle		
Stop Solution	1 bottle	Dilute sulfuric acid. Use directly according to the use volume. Pay attention to safety when using	

ASSAY PROCEDURE

1. Plate Set-up

- Bring all reagents to room temperature (22-28℃) equilibration (at least 30 minutes) before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.
- Determine the number of wells for the assay run. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
- Add 300 µL 1×Wash Buffer to each well and let stand for about 2 minutes. Aspirate or dump the liquid and pat dry on a paper towel, wash twice in this way.

2. Incubation with control and samples [Volume: 100 µL Time: 60 minutes]

- Add 100 µL antibody control or your test samples per well. The samples can be gradient diluted from 1:1000.
- Cover/seal the plate and incubate for 60 minutes at room temperature.
- Add 300 µL 1×Wash Buffer to each well and let stand for about 2 minutes. Aspirate or dump the liquid and pat dry on a paper towel, wash wells 3 times in this way. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. Incubation with Secondary Antibody [Volume: 100 µL Time: 60 minutes]

- Dilute HRP-Rabbit anti-Mouse IgG at 1:100 in 1×Dilution Buffer. Add 100 µL into each well, mix gently.
- Cover/seal the plate and incubate for 60 minutes at room temperature.
- Removal the liquid in the wells and repeat the aspiration/wash as in Step 2**.**

4. Incubation with Substrate

- Add 100 µL of Substrate Solution to each well, mix gently.
- Incubate for 20 minutes at room temperature**. Protect from light.** (According to the color of sample and the control antibody, the chromogenic time should be shortened or prolonged.)

5. Stop reaction

- Add 100 µL of Stop Solution to each well.
- Tap gently the plate to ensure it is well mixed.

6. Absorbance Reading

- Read absorbance of the entire plate at 450nm wavelength within 10 minutes after adding the stop solution.

INTERPRETATION OF RESULT

Positive index: Calculate the net OD(OD_{450nm}-Blank) mean+2SD of the negative samples.

Notes: It is suggested that each laboratory establish its own reference scope.

Antibody titer determination: the test sample results are determined as the maximum dilution when positive.

PROPERTIES OF PRODUCTS

Precision: Intra-assay precision CV% < 15%. Inter-assay precision CV% < 15%.

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the application which shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

1. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risks.
2. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
3. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

1. Bring all reagents and samples to room temperature before use.
2. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.
3. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
4. Read the absorbance of each well within 10 minutes after adding the stop solution.