



## **ELISA PRODUCT INFORMATION & MANUAL**

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

***NBP3-21466***

***Sample Insert for Reference Only***

Enzyme-linked Immunosorbent Assay for quantitative  
detection. For research use only.

Not for diagnostic or therapeutic procedures.

**Assay range: 7.81-500 pg/mL**

**Sensitivity: 1.7 pg/mL**

**Intended Use:** The kit has been verified by high purity SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India Protein. The use of this kit for natural samples need to be validated by the end user due to the complexity of natural targets and unpredictable interference.

## PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the Sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The 96-well strip plate is precoated with a monoclonal antibody specific for SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India. Standards and SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India present in the sample is bound by the immobilized antibody. After washing, a horseradish peroxidase conjugated anti- SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody -enzyme reagent, a TMB substrate solution is added to the wells and color develops in proportion to the amount of SARS-CoV-2 Spike RBD - Delta Variant, B.1.617.2, India bound in the initial step. The color development is stopped and the intensity of the color is measured at 450 nm.

## KIT CONTENTS AND STORAGE

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

Components	Amt	Preparation instructions	storage
Pre-coated microplate	1plate (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°C until expiration date and opened package store at 2-8°C for one month.
Detection Antibody	1 vial	Dilute at 1:1300 with 1×dilution buffer for 10 minutes before use. <b>Dilute fresh as needed.</b>	Primary liquid are stable at 2 - 8°C until expiration date. To be reconstituted, the working fluid is used within the working day and discard. So dilute fresh as needed.
Standard	1 bottle	Add 1 mL of 1×Dilution Buffer to the lyophilized standard bottle, briefly vortex to mix completely and prepare a standard stock solution.	
20 × Dilution Buffer	1 bottle	If crystals have formed in the 20 ×concentrated solution, bring to room temperature and mix until dissolved. Dilute the 20× concentrated solution to 1× working solution with deionized water. For example, make 400 ml of 1× Wash Buffer by adding 20 mL of 20× Wash Buffer to 380 mL of deionized water. <b>Dilute fresh as needed.</b>	
20 × Wash Buffer	1 bottle		
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, <b>DO NOT USE</b>	
Color Reagent B	1 bottle		
Stop Solution	1 bottle	Dilute acid. Use directly according to the use volume. Pay attention to safety when using	

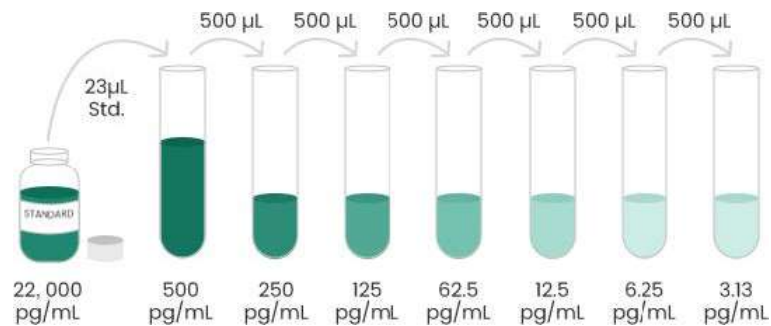
## ASSAY PROCEDURE

### 1. Plate Set-up

- Bring all reagents to room temperature (22-28°C) 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  $\mu\text{L}$  1 $\times$  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 standard and samples [Volume: 100 $\mu\text{L}$ Time: 2 hours]

- Make standard curve: Prepare 1000  $\mu\text{L}$  of the 500  $\text{pg}/\text{mL}$  top standard by adding 23  $\mu\text{L}$  of the standard stock solution in 977  $\mu\text{L}$  of 1 $\times$  Dilution Buffer. Perform six two-fold serial dilutions of the 500  $\text{pg}/\text{mL}$  top standard in 6 separate tubes using 500  $\mu\text{L}$  1 $\times$  Dilution Buffer as the diluents: after mixing the 500  $\text{pg}/\text{mL}$  top standard, pipette 500  $\mu\text{L}$  into the next tube, and so on. 1 $\times$  Dilution Buffer serves as the zero standard (0  $\text{pg}/\text{mL}$ ). Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.



- Add 100  $\mu\text{L}$  standard and your test samples per well. Cover/seal the plate and incubate for 2 hours at room temperature.
- Add 300  $\mu\text{L}$  1 $\times$  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 $\mu\text{L}$ Time: 1 hour]

- Add 100  $\mu\text{L}$  of detection antibody working solution into each well, mix gently.
- Cover/seal the plate and incubate for 1 hour at room temperature.
- Removal the liquid in the wells and repeat the aspiration/wash as in Step 2.

### 4. Incubation with Substrate [Volume: 100 $\mu\text{L}$ Time: about 20 minutes]

- Add 100  $\mu\text{L}$  of Substrate Solution (the mixture of Color Reagents A and B) 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

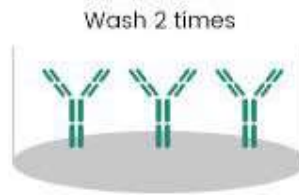
- Add 100  $\mu\text{L}$  of Stop Solution to each well.
- Tap gently the plate to ensure it is well mixed.

### 6. Absorbance Reading

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

## ASSAY PROCEDURE SUMMARY

1. Plate set-up



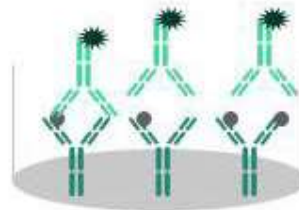
2. Incubation with standard and samples

Add 100  $\mu$ L standards or samples  
Incubate 2 hours, RT



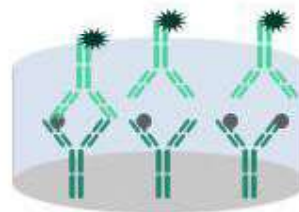
3. Incubation with Secondary Antibody

Wash 3 times  
Add 100  $\mu$ L Detection Antibody solution  
Incubate 1 hour, RT



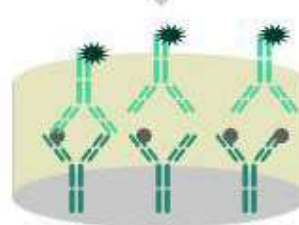
4. Incubation with Substrate

Wash 3 times  
Add 100  $\mu$ L Substrate Solution  
Incubate 20 min, RT, in the dark



5. Stop reaction

Add 100  $\mu$ L Stop Solution



6. Absorbance Reading

Read absorbance at 450nm  
within 10 minutes

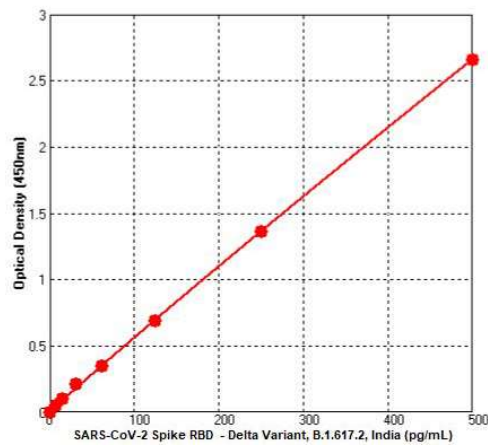
## CALCULATION OF RESULTS

1. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
2. Calculate the mean absorbance for each standard and sample, subtract average zero standard optical density.
3. The data been calculated by 4-parameter logistics curve-fitting algorithm.

## TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be run with each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
7.8125	0.044
15.625	0.096
31.25	0.206
62.5	0.341
125	0.687
250	1.357
500	2.657



## PERFORMANCE CHARACTERISTICS

### Precision:

Intra-assay Precision (Precision within an assay) - Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) - Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

Sample	Intra -assay Precision			Inter -assay Precision		
	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	91	180	363	83	179	367
SD	3.010	4.628	11.789	1.65	1.92	5.88
CV (%)	3.3%	2.6%	3.2%	2.0%	1.1%	1.6%

**Recovery:** The recovery of SARS-CoV-2 Delta (B.1.617.2) variant Spike RBD spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
Cell culture supernates (n=3)	92	88-96%

**Linearity:**

		Cell culture supernates
1:2	recovery of detected	107%
1:4	recovery of detected	98%
1:8	recovery of detected	104%
1:16	recovery of detected	99%

**Sensitivity:** 1.7 pg/mL. Which was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

**Specificity:** This assay recognizes the following factors:

Recombinant Protein	SARS-CoV-2 Lineage
SARS-CoV-2 Spike S1-His Recombinant Protein	Lineage A (Wuhan-Hu-1)
SARS-CoV-2 Spike S1+S2 ECD-His Recombinant Protein	
SARS-CoV-2 Spike S1-His Recombinant Protein	
SARS-CoV-2 Spike S1(D614G)-His Recombinant Protein, HPLC-verified	Abundant mutations in many lineages.
SARS-CoV-2 Spike S1(HV69-70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H)-His Recombinant Protein	B.1.1.7 (Alpha)
SARS-CoV-2 Spike RBD(N501Y)-His Recombinant Protein	
SARS-CoV-2 Spike S1 (L18F, D80A, D215G, LAL242-244 deletion, R246I, K417N, E484K, N501Y, D614G) Protein (His Tag)	B.1.351 (Beta)
SARS-CoV-2 Spike RBD(K417N, E484K, N501Y)-His Recombinant Protein	
SARS-CoV-2 Spike S1 (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y) Protein (His Tag)	P.1 (Gamma)
SARS-CoV-2 Spike RBD (K417T, E484K, N501Y) Protein (His Tag)	
SARS-CoV-2 Spike S1 (T95I, G142D, E154K, L452R, E484Q, D614G, P681R) Protein (His Tag)	B.1.617.1 (Kappa)
SARS-CoV-2 Spike RBD (L452R, E484Q) Protein (His Tag)	B.1.617, B.1.617.1 (Kappa), B.1.617.3
SARS-CoV-2 Spike S1 (T19R, G142D, E156G, 157-158 deletion, L452R, T478K, D614G, P681R) Protein (His Tag)	B.1.617.2 (Delta)
SARS-CoV-2 Spike RBD (L452R,T478K) Protein (His Tag)	
SARS-CoV-2 Spike S1 (W152C, L452R, D614G) Protein (His Tag)	B.1.429 (Epsilon)
SARS-CoV-2 Spike RBD(L452R)-His Recombinant Protein	
SARS-CoV Spike/S1 Protein (S1 Subunit, His Tag)	
SARS-CoV Spike S1+S2 ECD-His Recombinant Protein (S577A, Isolate Tor2)	

The assay couldn't recognize the following factors:

Recombinant Protein	SARS-CoV-2 Lineage
SARS-CoV-2 Spike RBD - Omicron Variant, B.1.1.529 Protein (His Tag)	B.1.1.529 (Omicron)

The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity and interference. No cross-reactivity and significant interference were observed.

<b>Recombinant Protein</b>
MERS-CoV Spike/S1 Protein (S1 Subunit, aa 1-725, His Tag)
Human coronavirus HKU1 (isolate N5) (HCoV-HKU1) Spike Protein (S1 Subunit, His Tag)
Human coronavirus HKU1 (isolate N1) (HCoV-HKU1) Spike/S1 Protein (S1 Subunit, His Tag)
Human coronavirus (HCoV-229E) Spike/S1 Protein (S1 Subunit, His Tag)
Human coronavirus (HCoV-NL63) Spike/S1 Protein (S1 Subunit, His Tag)
Human coronavirus (HCoV-OC43) Spike Protein (S1 Subunit, His Tag)
Influenza A H1N1 (A/California/07/2009) Hemagglutinin / HA Protein (His Tag)
Influenza A H1N1 (A/Michigan/45/2015) Hemagglutinin / HA Protein (His Tag)
Influenza A H3N2 (A/Switzerland/9715293/2013) Hemagglutinin / HA Protein (His Tag)
Influenza A H3N2 (A/Texas/50/2012) Hemagglutinin / HA Protein (His Tag)
Influenza A H5N1 (A/Hong Kong/483/1997) Hemagglutinin / HA Protein (His Tag)
Influenza A H7N9 (A/Anhui/1/2013) Hemagglutinin / HA Protein (His Tag)
Influenza B (B/PHUKET/3073/2013) Hemagglutinin / HA Protein (His Tag)
Influenza B (B/Brisbane/60/2008) Hemagglutinin / HA Protein (His Tag)
MERS-CoV Spike Protein (S1+S2 ECD, aa 1-1297, His Tag)
Human coronavirus (HCoV-NL63) Spike Protein (S1+S2 ECD, His Tag)
Human coronavirus (HCoV-229E) Spike Protein (S1+S2 ECD, His Tag)
Human coronavirus HKU1 (isolate N5) (HCoV-HKU1) Spike Protein (S1+S2 ECD, His Tag)
Human coronavirus (HCoV-OC43) Spike Protein (S1+S2 ECD, His Tag)

## **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.

## **TECHNICAL 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.