



## Technical Manual

# SARS-COV-2 Spike RBD Protein IgG Antibody ELISA Kit

- **Catalogue Code: CBK4137**
- **Qualitative ELISA Kit**
- **Research Use Only**

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## Introduction

This kit is used for the qualitative detection of the level of anti-SARS-CoV-2 (2019-nCoV) Spike S1 Protein IgG antibodies in serum and plasma. Only used for qualitative tests and not quantitative studies.

## Principle of the Assay

This assay employs the Indirect immunoassay technique. A SARS-COV-2 Spike RBD Protein LgG Antibody has been pre-coated onto a microplate. Antibodies and samples are pipetted into the wells and any Spike S1 Protein Antibody present is bound by the immobilized protein. Following incubation, unbound samples are removed during a wash step. A secondary antibody is then added to the wells and binds to the combination of capture protein-Spike S1 Protein Antibody in the sample. Following a wash to remove any unbound combination, a substrate is added. A colored product TMB is formed in proportion to the amount of Spike S1 Protein Antibody present in the sample. The reaction is terminated by the addition of acid and the absorbance is measured.

## Materials Provided

Unopened Kit: Store at 2-8°C and the kit is stable for 6 months upon receipt. Do not use past expiration date. It is highly recommended to use the remaining agents within 1 month provided this is prior to the expiration date of the kit.

Part	Size (96T)	Storage of opened/ Reconstituted material
<b>Antigen Coated Plate</b>	8 x 12	Return unused wells to the foil pouch containing the desiccant pack and store at 2-8°C. Reseal along the entire edge of the zip-seal.
<b>Control Antibody (100x)</b>	1x 20ul	Aliquot and store at ≤ -20°C in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
<b>Concentrated Secondary Antibody (1000x)</b>	1 x30ul	May be stored for up to 6 months at 2-8°C.*
<b>Control/Sample Diluent (R1) (4x)</b>	1 x20mL	May be stored for up to 6 months at 2-8°C.*
<b>Secondary Antibody Diluent (R2)</b>	1 x 12mL	
<b>Wash Buffer (20x)</b>	1 x 30mL	
<b>TMB Substrate</b>	1 x12 mL	
<b>Stop Solution</b>	1 x6 mL	
<b>Plate Sealers</b>		4 strips
<b>Specification</b>		1

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## Other materials required but not supplied:

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630nm or 570nm.
2. Pipettes and pipette tips
3. Deionized or distilled water
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator
6. Test tubes for dilution of standards and samples.

## Precautions

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature can cause variation in binding.
2. Variations in sample collection, processing, and storage may cause sample value differences.
3. Reagents may be harmful and if ingested, rinse with an excess amount of tap water.
4. Stop Solution contains strong acid. Wear eye, hand, and face protection.
5. Please perform simple centrifugation to collect the liquid before use.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. Adequate mixing is required for good results. Use a mini-vortex at the lowest frequency.
8. Mix the sample and all components in the kits adequately and use clean plastic container to prepare all of the diluent.
9. Both the sample and standard should be assayed in duplicate, and the sequence of the reagents should be added consistently.
10. Reuse of dissolved standard is not recommended.
11. The kit should not be used beyond the expiration date on the kit label.
12. The kit should be away from light when it is stored or incubated.
13. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
14. To avoid cross contamination, use disposable pipette tips.
15. Please prepare all the kit components according to the specifications. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months. 6
16. The 48T kit is also suitable for the specification.

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## Sample Collection and Storage:

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing the corrected IgG as in this kit may interfere with this assay.

**Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid freeze/thaw cycles.

**Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection and detect. Assay immediately or aliquot and store samples at -20°C. Avoid freeze / thaw cycles. (Note: Citrate plasma has not been validated for use in this assay)

**Notes:** It is suggested that all samples in this study be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.

## Reagent Preparation

**Bring all reagents to room temperature before use. If crystals have formed in the concentrate, bring the reagent to room temperature, and mix gently until the crystals have completely dissolved.**

**Control/Sample Diluent (R1) (4X):** Dilute 1:4 with double distilled or deionized water before use, for example: Add 5mL of Control/Sample Diluent Concentrate to 15mL of deionized or distilled water to prepare 20mL of Control/Sample Diluent.

**Control Antibody:** Dilute 1:100 with the 1xControl/Sample Diluent(R1), sit for a minimum of 15 minutes with gentle agitation.

**Concentrated Secondary Antibody (1000x):** Dilute 1:1000 with the Secondary Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

**Wash buffer:** : If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 1:20 with double distilled or deionized water before use, for example: add 20mL of Wash Buffer Concentrated to 380mL of deionized or distilled water to prepare 400mL of Wash Buffer.

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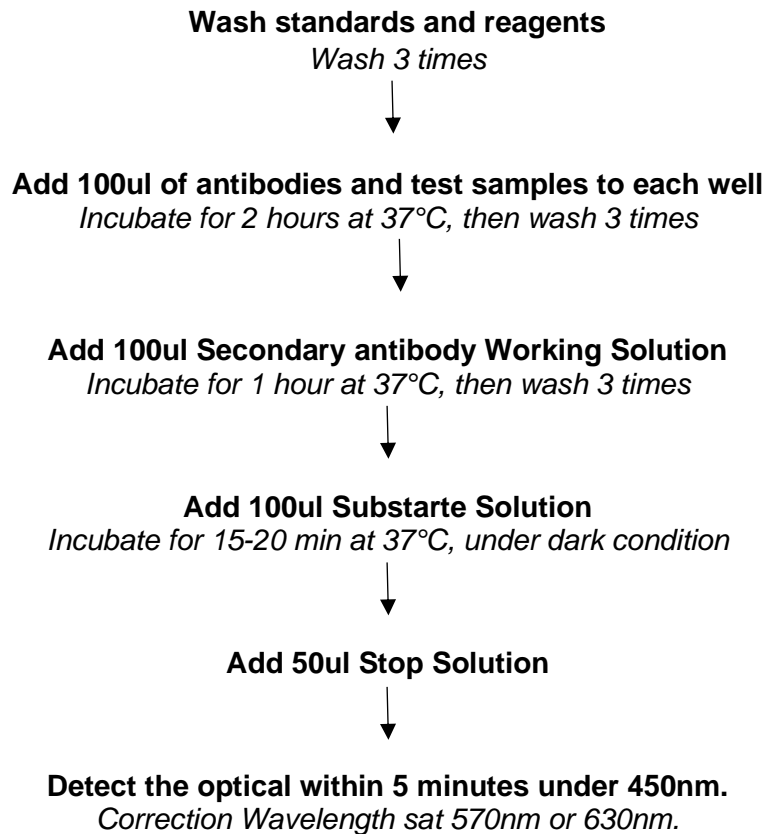
## Assay Procedure

Bring all reagents to room temperature before use. It is recommended that all standards, controls and samples be assayed in duplicate.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 350  $\mu\text{L}$ /well, aspirate each well after holding 60 seconds. Repeat the process four times for a total of five washes.
3. Add 100  $\mu\text{L}$  1xControl/Sample Diluent (R1) in blank well.
4. Add 100  $\mu\text{L}$  Control Antibody Working Solution and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
5. Repeat the aspiration/wash as in step 2.
6. Prepare the Concentrated Secondary Antibody (1000X) Working Solution 15 minutes early before use.
7. Add Secondary Antibody Working Solution in each well (100 $\mu\text{L}$ /well), cover with the new adhesive strip provided. Incubate for 1 hour at 37°C.
8. Warm-up the Microplate reader for at least 30 minutes before use.
9. Repeat the aspiration/wash as in step 2.
10. Add TMB Substrate (100 $\mu\text{L}$ /well). Incubate for 15-20 minutes at 37°C. Protect from light.
11. Add Stop Solution (50 $\mu\text{L}$ /well), determine the optical density of each well within 5 minutes using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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## Assay Procedure Summary



## Specificity

This assay recognizes SARS-COV-2 Spike RBD Protein IgG Antibody.

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## Precision

### Intra-plate Precision

3 samples with low, middle and high levels SARS-COV-2 Spike RBD Protein IgG Antibody were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

### Inter-plate Precision

3 samples with low, middle and high levels SARS-COV-2 Spike RBD Protein IgG Antibody were tested 20 times on different plates, 8 replicates in each plate.

Inter-Assay: CV<15%

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
<b>N</b>	20	20	20	20	20	20
<b>Mean (pg/mL)</b>	12	51	190	30	50	195
<b>Standard Deviation</b>	0.2	2.3	3.7	1.6	2.3	6.7
<b>CV(%)</b>	1.7	4.5	1.9	5.3	4.6	3.4



## Trouble Shooting

Problem	Cause	Solution
High Background	Insufficient washing	Sufficiently wash plate as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate	Warm the reader up before use. Make sure to set up appropriate main wavelength and correct wavelength
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.
	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.
	Matrix effect of samples	Use positive control
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiment.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitate in samples	Centrifuge samples before use
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.

\*For research use only. Not for therapeutic or diagnostic purposes.

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Notes:

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### **Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

### **Contact Details**



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