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Data sheet
SARS-CoV-2 IgG Detection Kit (Colorimetric Anti-Spike S1 RBD IgG detection)

Catalog #79985
Size: 96 reactions

DESCRIPTION: The SARS-CoV-2 IgG detection kit is designed for qualitative detection of human IgG antibodies in serum collected from individuals suspected of prior infection with the virus that causes COVID-19. This fast and simple ELISA uses the receptor binding domain (RBD) of the SARS-CoV-2 Spike protein (BPS Bioscience #100696) to identify IgG antibodies that indicate a previous infection with SARS-CoV-2. The SARS-CoV-2 IgG (Colorimetric Anti-Spike S1 RBD IgG detection) Detection Kit comes in a convenient 96-well format, with purified SARS-CoV-2 RBD protein and an HRP-conjugated anti-human Fc antibody for 100 binding reactions.

BACKGROUND: The pandemic coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As a first step of the viral replication strategy, the virus attaches to the host cell surface before entering the cell. The Spike protein recognizes and binds to the Angiotensin-Converting Enzyme 2 (ACE2) receptor found on the surface of type I and II pneumocytes, endothelial cells, and ciliated bronchial epithelial cells. Drugs targeting the interaction between the Spike protein of SARS-CoV-2 and ACE2 may offer some protection against the viral infection.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100696	Spike S1 RBD, Avi-His tag (SARS-CoV-2)*	5 µg	-80°C	Avoid multiple freeze/ thaw cycles!
	anti-human Fc-HRP conjugate (1 mg/ml)	5 µl	+4°C	
	Colorimetric HRP substrate	10 ml	+4°C	
79964	Transparent 96-well microplate	1	Room Temp	

**The initial concentration of Spike RBD is lot-specific and will be indicated on the tube containing the protein.*

APPLICATIONS: This kit is useful for detection of anti SARS-CoV-2 Spike S1 RBD IgG in human serum

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

REFERENCES: Long, Q.-X., *et al*. 2020. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat. Med.* (in press). https://doi.org/10.1038/s41591-020-0897-1

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

PBS (Phosphate buffered saline)
PBST (Phosphate buffered saline containing 0.05% Tween-20)
Dry Milk (Fisher #115668 or compatible)
1N HCl (aqueous)
Rotating or rocker platform
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Coating the plate with SARS-CoV-2 Spike S1 RBD protein:

1) Thaw **Spike S1 RBD protein** on ice. Upon first thaw, briefly spin tube containing **Spike S1 RBD protein** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **Spike S1 RBD protein** in aliquots at -80°C. Note: **Spike S1 RBD protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **Spike S1 RBD protein** to 1 µg/ml in PBS.
3) Add 50 µl of diluted **Spike S1 RBD protein** solution to each well and incubate at 4°C overnight.
4) After the overnight coating, discard the solution and wash the plate once with 200 µl PBST. Tap plate onto clean paper towels to remove liquid.
5) Block wells by adding 200 µl blocking buffer (PBST containing 5% dry milk) to each well. Incubate for 1 hour at room temperature with slow shaking. Remove the blocking solution and wash the plate twice with 200 µl PBST. Tap plate onto clean paper towels to remove liquid.

Serum sample dilution, IgG capture and detection

1) Dilute the serum sample 1:50 in the blocking buffer (PBST containing 5% dry milk) and add 50 µl diluted sample to the test well.
2) For the “Negative Control,” add 50 µl of diluted serum that was collected be from a non-Covid19 patient. For the “Blank,” add 50 µl of the blocking buffer only. Incubate at room temperature for one hour with slow shaking.
3) After one hour, discard the solution and wash the plate three times with 200 µl PBST. Tap plate onto clean paper towels to remove liquid.
4) Dilute **anti-human Fc-HRP conjugate** in the blocking buffer (PBST containing 5% dry milk) at 1:2000. Add 50 µl of diluted anti-human Fc-HRP conjugate to each well, and incubate at room temperature for 30 minutes.

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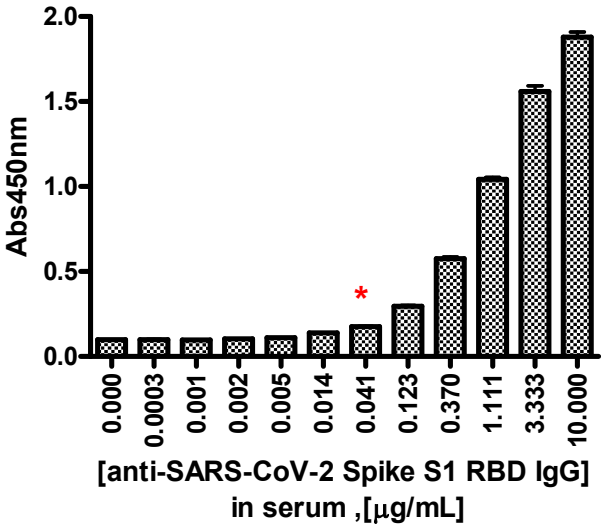
5) After 30 minutes, discard the solution and wash the plate three times with 200 µl PBST. Tap plate onto clean paper towels to remove liquid.
6) Prepare the stop solution (1N HCl in water). Note: 2N H_2SO_4 or other compatible acidic solutions can be substituted.
7) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the positive control well. This usually takes ~ 1 minute but could take less than a minute depending on the IgG level in the sample serum. The optimal incubation time may vary, and should be determined empirically by the user. It is recommended that the reaction be stopped when the 'Negative Control' well is lower than ~ 0.1 absorbance at 450 nm.
8) Once blue color has developed in the positive well, add 100 µl stop solution prepared in step 6. The positive well should become yellow.
9) Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader. "Blank" value is subtracted from all readings. The blank wells should exhibit an absorbance of ~ 0.1 or less at 450 nm as noted above.

Example of assay results:



Anti-SARS-CoV-2 Spike S1 RBD IgG in serum was detected by using **SARS-CoV-2 IgG Detection Kit (Colorimetric Anti-Spike RBD S1 IgG detection)**. Positive control human anti-Spike (IgG1) was added to COVID-19 negative human serum at indicated concentrations, and diluted in the blocking buffer as described in the protocol. ~ 50 ng/ml of IgG in serum can be detected with confidence (absorbance should be at least 1.5 fold higher than the negative serum; e.g. $Abs_{Negative\ Control}$ = 0.1, $Abs_{41ng/ml}$ = 0.17, $Abs_{123ng/ml}$ = 0.3)

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RELATED PRODUCTS:

Product Name	Catalog#	Size
SARS-CoV-2 Spike:ACE2 Inhibitor Screening Assay Kit	79931	96 reactions
ACE2:SARS-CoV-2 Spike Inhibitor Screening Assay Kit	79936	96 reactions
ACE2:SARS-CoV-2 Spike S1-Biotin Inhibitor Screening Assay Kit	79945	96 reactions
SARS-CoV-2 Spike S1-Biotin:ACE2 TR-FRET Assay Kit	79949	96 reactions
Spike S1, Fc Fusion, Avi-tag (SARS-CoV-2)	100678	100 µg/1 mg
Spike S1, Fc fusion, Avi-tag, Biotin-Labeled (SARS-CoV-2)	100679	25 µg/50 µg
Spike S1 RBD, His-tag (SARS-CoV-2)	100687	50 µg/100 µg
Spike S1, Fc fusion (SARS-CoV-2)	100688	20 µg/50 µg
Spike S1 RBD, Fc fusion (SARS-CoV-2)	100699	50 µg/100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
ACE2, His-tag	11003	20 µg/100 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665	20 µg/50 µg
ACE2, Fc Fusion (Monkey)	100701	50 µg/1 mg
ACE2, His-tag (Monkey)	100702	50 µg/1 mg

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TROUBLESHOOTING GUIDE:

Problem	Possible cause	Solution
Colorimetric signal of positive control reaction is weak	Spike trimer or serum sample has lost activity	Increase time of incubation with serum. Increase serum concentration. Proteins lose activity upon repeated freeze/thaw cycles. Store S1 trimer and anti-Fc HRP conjugate in single-use aliquots.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Colorimetric signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the wells
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of proteins to create a standard curve

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