Data sheet
SARS-CoV-2 IgG Detection Kit
(Colorimetric Trimer Anti-Spike IgG detection)
Catalog #AMS.79975
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 trimeric form of the SARS-CoV-2 Spike protein (#AMS.100728) to identify IgG antibodies that indicate a previous infection with SARS-CoV-2. The Spike protein is expressed on the viral membrane as a trimer, which means this kit measures IgG antibodies in a more physiologically relevant context than many other commercially available ELISA kits. The SARS-CoV-2 IgG Detection Kit comes in a convenient 96-well format, with purified trimeric SARS-CoV-2 Spike protein and an HRP-conjugated anti-human Fc antibody for 100 binding reactions.

BACKGROUND: As the viral load increases in the infected individual prior to the onset of symptoms, the individual may unknowingly be able to actively spread the infection during this presymptomatic phase. Once the immune system recognizes the infection, IgM is generated against the virus initially, followed by a second response leading to the production of higher affinity IgG molecules targeting the SARS-CoV-2 virus. IgG antibodies to SARS-CoV-2 generally become detectable beginning 10 – 14 days following infection but may occur later. The presence of IgG antibodies following previously negative testing defines IgG antibody seroconversion following SARS-CoV-2 infection. False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. This assay kit is not intended for clinical diagnostic use.

COMPONENTS:

Catalog #	Component	Amount	Storage	
AMS.100728	Spike Trimer (S1 + S2), 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	
	Transparent 96-well microplate	1	Room Temp	

APPLICATIONS: This kit is useful for detection of anti SARS-CoV-2 Spike 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

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 Trimeric SARS-CoV-2 Spike protein:

1) Thaw **Spike protein** on ice. Upon first thaw, briefly spin tube containing **Spike protein** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **Spike protein** in aliquots at -80°C. Note: **Spike protein** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **Spike protein** to 1 µg/ml in PBS.
3) Add 50 µl of diluted **Spike protein** solution to each well and incubate at 4^{o}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 and add 50 µl diluted sample to the test well.
2) For the “Negative Control”, add 50 µl of diluted serum that was collected from a non-Covid19 patient. For the “Blank”, add 50 µl of only the blocking buffer . 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.

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.

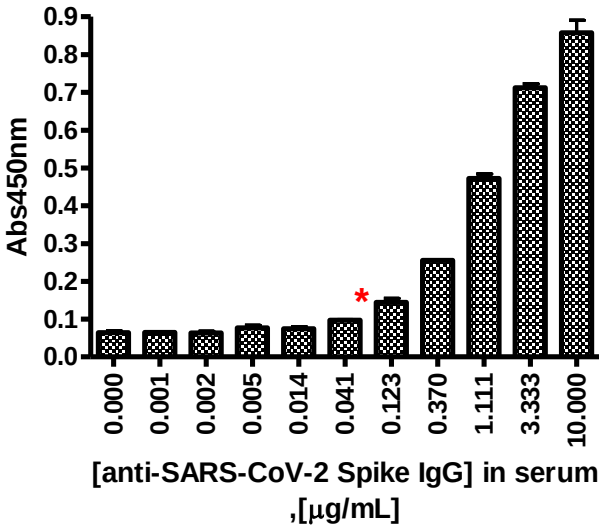
6) Prepare the stop solution (1N HCl in water). Note: 2N H_2SO_4 or other compatible acidic solutions can be substituted.

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 appear 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 IgG in serum was detected by using **SARS-Cov-2 IgG detection kit**. 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. ~ 100 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. $Abs_{Negative\ Control}$ = 0.06, $Abs_{41ng/ml}$ = 0.1, $Abs_{123ng/ml}$ = 0.14)

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