AffinityImmuno®

SARS-CoV-2 virus neutralization assay (spike protein/ ACE2 ligand binding assay)
Catalog EL-1611-32214

For the quantitative determination of inhibition of SARS-CoV-2 spike protein binding to ACE2.

Introduction

The emergence of the COVID-19 pandemic resulting from the spread of the SARS-CoV-2 virus has ignited a massive global research effort for development of COVID-19 vaccines, therapeutics, and diagnostic tests for viral infections and antibody responses. These time-critical efforts have also put into focus the need for better approaches for immune response analysis in both the human population exposed to the wild virus and in vaccine studies. In addition to population monitoring and vaccine studies, ligand binding assays may be of use in library screens to identify monoclonal antibodies, peptides, and small molecules, that may block infection or lower the infection rate of SARS-CoV-2 virus

To keep step with these rapidly developing efforts, AffinityImmuno Inc.'s SARS-CoV-2 spike protein ligand binding assay allows rapid quantification of COVID-19 neutralizing antibodies in serum that block the interaction of spike protein with its receptor ACE2. This assay allows rapid bioanalysis of antibody-based therapeutics, vaccine leads, and immune response to the virus in the human population.

Each kit includes:	Units
Coated microtiter plate, 96 wells (1x8 strips)	1
Ready-to-use Calibrator Samples - Human serum samples with calibrated blocking activity	Calibrator 1 (250µl) 0% inhibition Calibrator 2 (250µl) 20% inhibition Calibrator 3 (250µl) 40% inhibition Calibrator 4 (250µl) 60% inhibition Calibrator 5 (250µl) 80% inhibition Calibrator 6 (250µl) 100% inhibition
Do not mix or substitute reagents with those from other lots.	

Each kit includes:	Units
1X assay buffer	50ml
10X wash buffer	50µl
100X detection reagent	150µl
TMB	12ml
TMB stop solution	12ml
Plate sealers	3
Do not mix or substitute reagents with those from other lots.	

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

Safety precautions

- The test protocol must be followed strictly.
- All reagents containing serum should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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Preparation of reagents

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation:** Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 50mL concentrate to 450mL ultra-pure water). Mix well.
2. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay/wash buffer 1/100 before use (for example add 150µl concentrate to 15ml of assay buffer). Mix well.

Specimen storage

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

Assay procedure

1. Remove kit from -20ºC and allow precoated plate to acclimate to room temperature for 15 to 20 minutes. Thaw all other components on ice.
2. Apply 100µl of the ready-to-use Calibrator Samples to the appropriate wells in duplicate.
3. Dilute each test sample by adding an equal volume of assay buffer. For example, add 100µl assay buffer to 100µl of test sample.
4. Add 80µl test sample to each well for testing.
5. Add 20µl diluted detection reagent to each well of test sample.
6. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
7. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
8. Add 100µL of TMB to each well on plate. Incubate for 3-5 minutes at room temperature protected from light.
9. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
10. Determine absorbance with a microplate reader at 450nm against 620nm.

Calculations and results

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
2. We recommend each lab develop their own statistical cutpoint using methodologies as described by G. Shankar, et al. (2008). (Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J. Pharmaceutical and Biomedical Analysis 48:1267–1281).

Performance characteristics

Precision: Intra-assay coefficient of variation (CV) < 10%. Inter-assay CV < 10%.

Ordering Information

Please visit www.afsbio.com to order this product, or contact us at info@afsbio.com.

If ordering online, your order will be processed immediately.

Materials and storage

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Sample data

Table 1. 8 serum samples collected in 2019 (pre-Covid) and >28 days after resolution of PCR confirmed SARS-CoV-2 infection analyzed using the above method.

Serum ID	Pre-Covid serum OD450	Covid serum OD450	Severity
1	1.256	0.467	Mild
2	1.277	1.046	N/A
3	1.304	0.130	Mild
4	1.552	0.589	Mild
5	1.364	0.697	Mild
6	1.304	0.139	Moderate
7	1.352	0.397	N/A
8	1.345	0.403	Mild

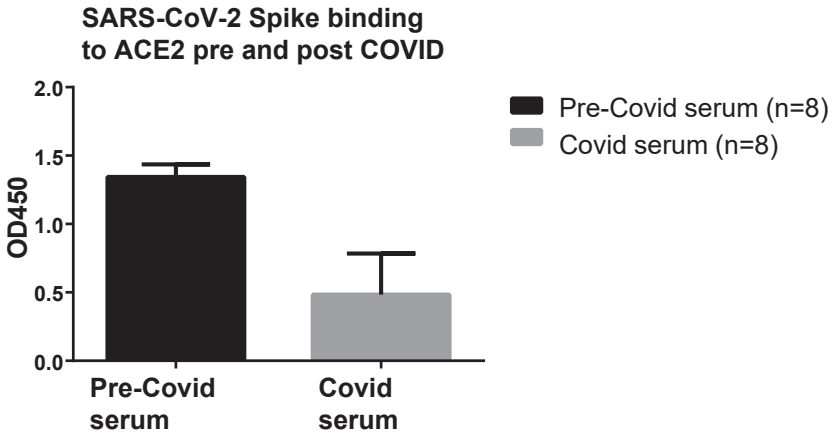

Figure 1. Graphic representation of the data in table 1.