SARS-CoV-2 surrogate Virus Neutralization Test (sVNT)(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 novel approaches to analyzing the antibody response in both the human population exposed to the wild virus and in vaccine studies. In addition to population monitoring and vaccine studies, virus neutralization testing may be used 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.

AffinityImmuno Inc.'s SARS-CoV-2 **surrogate Virus Neutralizing Test (sVNT)** allows rapid quantification of serum or plasma COVID-19 neutralizing antibodies by measuring blocking of the interaction between spike protein and its receptor ACE2. This assay allows analysis of antibody-based therapeutics, vaccine leads, and immune response to the virus in the human population in as little as 1.5 hours making it the most rapid screen for virus neutralizing antibodies.

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 (125µl) 0% inhibition Calibrator 2 (125µl) 20% inhibition Calibrator 3 (125µl) 40% inhibition Calibrator 4 (125µl) 60% inhibition Calibrator 5 (125µl) 80% inhibition Calibrator 6 (125µl) 100% inhibition
Do not mix or substitute reagents with those from other lots.	

Each kit includes:	Units
1X assay buffer	50mL
10X wash buffer	50mL
100X detection reagent	80µL
TMB	12mL
TMB stop solution	12mL
Do not mix or substitute reagents with those from other lots.	

Materials and instruments required but not supplied

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

Safety precautions

- The test protocol must be followed.
- 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.
- Disposal must be performed in accordance with local regulations.
- Only trained laboratory personnel should execute this test.

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

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 50 mL concentrate to 450 mL ultra-pure water). Mix well.
2. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay/wash buffer 1/100 before use (for example add 80µl concentrate to 7.92 mL of assay buffer). Mix well.

Specimen storage

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

Assay procedure

1. Remove the kit from -20ºC and allow precoated plate to acclimate to room temperature for 15-20 minutes. Thaw all other components on ice.
2. Add 40 µl calibrator or test sample (neat serum or neat plasma) to each well for testing.
3. Add 60 µl of prepared 1X Detection reagent to each well containing calibrator or test sample.
4. Incubate for 1 hour at room temperature on a plate shaker at approx 300 rpm.
5. Discard the content of the plate and wash the wells 3x with 200 µL wash buffer per well. Tamp the plate on stack of paper towel to remove excess liquid.
6. Add 100 µL of TMB to each well on plate. Incubate for 5-10 minutes at room temperature protected from light.
7. Add 100 µL of TMB stop solution to each well on plate.
8. Determine absorbance with a microplate reader at 450 nm against 620 nm.

Calculations and results

1. Because this is a competitive assay, strong signals indicate interaction between the detection reagent (SARS-CoV-2 spike protein S1 subunit) and its receptor, ACE2. In the presence of blocking antibodies this signal will be reduced depending on the concentration of the blocking antibody and the strength of binding.
2. 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.

Performance characteristics

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

Ordering Information

Please vist www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.

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. Mix and briefly centrifuge tubes on pulse setting prior to opening to ensure proper mixing.

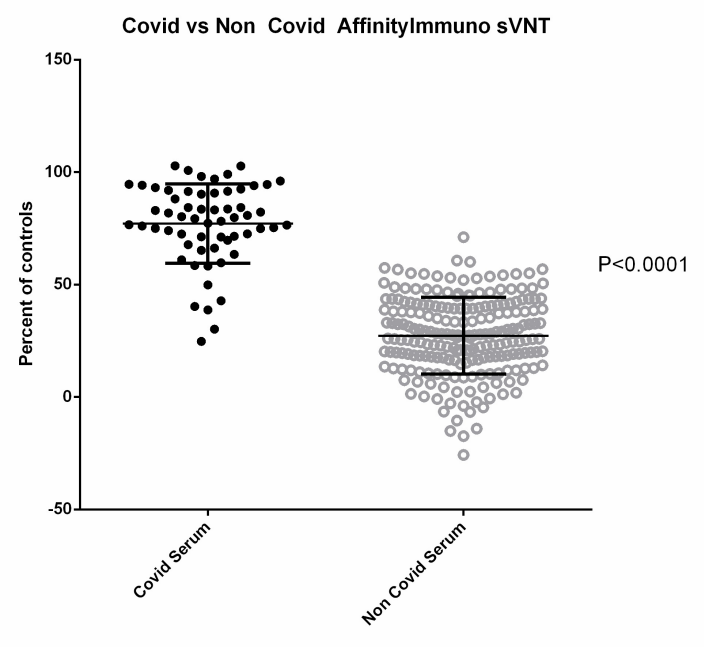

Figure 1. Comparison of 62 serum samples from recovered, COVID-19 individuals to 260 non-COVID-19 (self-declared) individuals demonstrates the presence of virus neutralizing antibodies that prevent binding between the spike protein and its receptor, ACE2.