GENLISA™ SARS-CoV-2 (Covid-19) Epsilon Neutralizing Antibody (B.1.429, B.1.427) ELISA

REF : KBVH440

Ver1.0

RUO

Enzyme Immunoassay for the Qualitative and Quantitative Detection of all types of Neutralizing Antibodies against SARS-CoV-2 (Variant B.1.429, B.1.427) in a species- and isotype-independent manner in serum or plasma using a S1 Protein in the kit

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBVH440 Σ 96 tests

KRISHGEN BioSystems For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: tel +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

Introduction:

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a blocking ELISA technique which mimics the virus neutralization process.

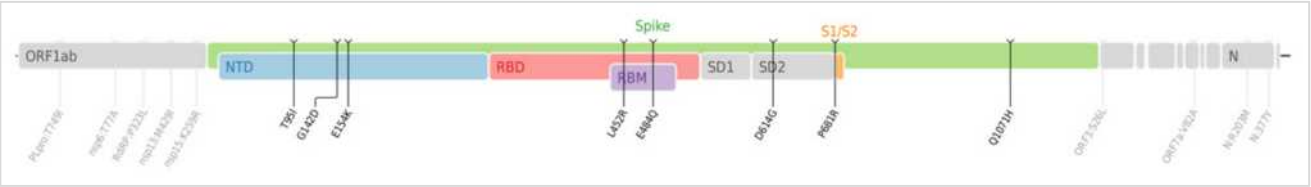
SARS-CoV-2-neutralizing antibodies primarily target the trimeric spike (S) glycoproteins on the viral surface that mediate entry into host cells. The S protein has two functional subunits that mediate cell attachment (the S1 subunit, existing of four core domains S1A through S1D) and fusion of the viral and cellular membrane (the S2 subunit).

Potent neutralizing antibodies often target the receptor interaction site in S1, disabling receptor interactions. The spike proteins of SARS-CoV-2 commonly bind to the human angiotensin coverting enzyme 2 (ACE2) protein as a host receptor through their S1B domain. Receptor interaction is known to trigger irreversible conformational changes in coronavirus spike proteins enabling membrane fusion.

SARS-CoV-2 initiates an immune response, which leads to the production of antibodies. These neutralizing antibodies provide protection against future infection from SARS-CoV-2 (Variant B.1.429, B.1.427), as they remain in the circulatory system for months to years post infection.

SARS-CoV-2 keeps evolving by continual mutation which enables the virus to evade vaccines and immune systems. Some of the mutations, the U.K. variant (B.1.1.7), the Brazil variant (P.1), the South Africa variant (B.1.351), the Indian variant (B.1.617), and the Omicron variant (B.1.1.529) may have allowed the virus to escape from neutralizing antibodies.

This Neutralizing Kit works on using Recombinant SARS-CoV-2 (2019-nCoV) Spike S1 Protein and a Neutralizing Antibody sensitive to the variant as a Standard/Calibrator to measure the effective inhibition %.



Intended Use:

The GENLISA™ SARS-CoV-2 (Covid-19) Epsilon Neutralizing Antibody (B.1.429, B.1.427) ELISA kit is used as an analytical tool for the qualitative and quantitative detection all types of neutralizing antibodies against SARS-CoV-2 spike S1 proteins in serum or plasma.

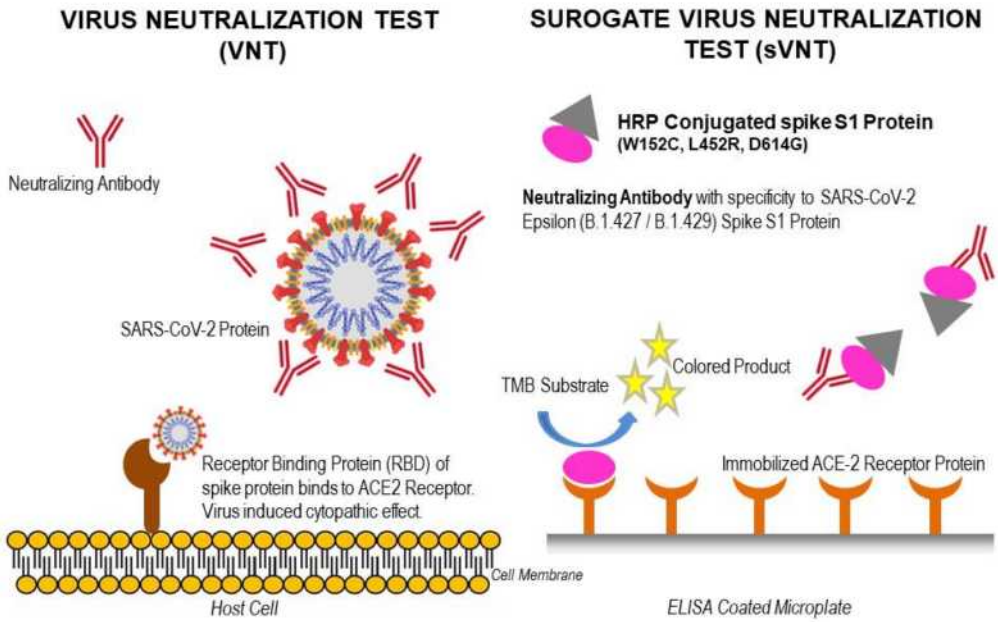
Principle:

The method employs sandwich ELISA technique. The protein-protein interaction between HRP-SARS-CoV-2 spike S1 and hACE2 can be blocked by neutralizing antibodies against SARS-CoV-2 spike S1.

Samples and controls are pipetted in a blank microtitre plate and incubated with HRP conjugated human SARS-CoV-2 spike S1 protein. The antibodies to SARS-CoV-2 present in the samples and controls bind to the SARS-CoV-2 spike S1 protein to form a complex.

This solution of bound and unbound antibodies to SARS-CoV-2 Variant (B.1.429, B.1.427) is then pipetted into human ACE2 coated microplate. After washing to remove the bound complex of Anti-SARS-CoV-2 Variant (B.1.427, B.1.429) and HRP conjugated SARS-CoV-2 spike S1, the substrate solution (TMB) is added to the microwells.

Post incubation, color develops proportionally to the amount of unbound Anti-SARS-CoV-2 (Covid-19) Variant (B.1.429, B.1.427) present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



PRINCIPLE OF THE GENLISA™ SARS-COV-2 (Epsilon Variant) surrogate Virus Neutralization Test / Neutralizing Antibody ELISA

Materials Provided:

Part	Description	Qty
Human ACE-2 Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Human ACE2 protein	1 x 96 wells
Blank Microtiter Plate	96 well polystyrene uncoated microplate (12 strips of 8 wells)	1 x 96 wells
Anti-Human SARS-CoV-2 (sensitized to Epsilon Variant B.1.429, B.1.427) Standard	Anti-Human SARS-CoV-2 (sensitized to Epsilon Variant B.1.429, B.1.427) Standard (lyophilized, concentrated - 2500 ng/ml)	2 vials
SARS-CoV-2 spike S1:HRP Conjugate	SARS-CoV-2 spike S1:HRP Conjugate prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with preservative thiomersol < 0.01%	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with preservative thiomersol < 0.01% with 1:1000 dilution human serum	10 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	2N Sulfuric Acid	12 ml
Instruction Manual		1 no

*note: the Anti-Human SARS-CoV-2 Standard is to be used as Positive Control when running a qualitative assay by preparing it as the highest standard of 2000 ng/ml and prepared accordingly when running a qualitative assay.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommend temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided.

Samples should be diluted 1:100 (v/v) for optimal recovery, (for example 1 ul sample + 99 ul sample diluent) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted less or more respectively with Sample Diluent accordingly.

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Preparation before Use:

Allow serum or plasma samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

In cases where matrix interferences is under or over observed, the samples may be diluted with Sample Diluent accordingly.

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. **Reconsitute the lyophilized standard in 1ml Standard Diluent to get a concentration of 2500 ng/ml**
4. To make **Wash Buffer (1X)**; dilute **25 ml** of **20X Wash Buffer** in **475 ml of DI water**.

For Quantitative Assay, dilute the concentrated Standard provided as under -

Standards Preparation: Perform serial dilutions by using reconstituted main stock standard solution as per the below table. Thus the Anti-SARS-CoV-2 (spike antibody) Standards concentration is 2000 ng/ml, 500 ng/ml, 125 ng/ml, 62.5 ng/ml, 31.25 ng/ml, 7.825 ng/ml and 0 ng/ml. Sample Diluent serves as the zero standard (0 ng/ml).

Standard Concentration	Standard No	Dilution Particulars
2500 ng/ml	Original Standard	Standard (lyophilized), reconstituted with 1 ml Standard Diluent
2000 ng/ml	Standard No.7	400 ul Original Standard + 100 ul Standard Diluent (1X)
500 ng/ml	Standard No.6	125 ul Standard No. 7 + 375 ul Standard Diluent (1X)
125 ng/ml	Standard No.5	125 ul Standard No. 6 + 375 ul Standard Diluent (1X)
62.5 ng/ml	Standard No.4	250 ul Standard No. 5 + 250 ul Standard Diluent (1X)
31.25 ng/ml	Standard No.3	250 ul Standard No. 4 + 250 ul Standard Diluent (1X)
7.825 ng/ml	Standard No.2	125 ul Standard No. 3 + 375 ul Standard Diluent (1X)
0 ng/ml	Standard No.1	500 ul Standard Diluent (1X)

note: the Anti-Human SARS-CoV-2 Standard is to be used as Positive Control when running a qualitative assay by preparing it as the highest standard of 2000 ng/ml and Standard Diluent as the Negative Control.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-Human SARS-CoV-2 (Covid-19).
3. It is recommended that the Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

A) Neutralization Reaction

1. Pipette **100 ul** of **Negative Control (marked as Standard No. 1)** in duplicate to the respective wells in the blank microplate.

2. Pipette **100 ul** of **Positive Control** or freshly prepared **Standards** (for quantitative assay) in duplicate to the respective wells in the blank microplate.

3. Pipette **100 ul** of the diluted **Samples solution** into the respective wells in the blank microplate.

4. Add **100 ul** of **SARS-CoV-2 spike S1:HRP Conjugate** into all the wells.

5. Seal the plate and **incubate** for **60 minutes** at **Room Temperature (18-25°C)**.

B) Binding Reaction

1. Pipette **100 ul** of **Negative Control solution** into the respective wells of the human ACE-2 coated microplate from the neutralization reaction plate.

2. Pipette **100 ul** of the **Positive Control solution** or freshly prepared **Standards solution** (for quantitative assay) into the respective wells of the human ACE-2 coated microplate from the neutralization reaction plate.

3. Pipette **100 ul** of the diluted **Samples solution** into the respective wells of the human ACE-2 coated microplate from the neutralization reaction plate.

4. Seal plate and **incubate** for **90 minutes** at **Room Temperature (18-25°C)**.

5. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

6. Pipette **100 ul** of **TMB Substrate solution**.

7. **Incubate** in the dark for **30 minutes** at **Room Temperature**.

8. Stop reaction by adding **100 ul** of **Stop Solution** to each well.

9. **Read Absorbance** at 450 nm within 30 minutes of stopping reaction.

Qualitative Interpretation:
Calculation for Cut Off Values -
Read the sample and negative/positive control wells on microtitre plate reader at 450nm.

The OD (Optical Density) of NC (Negative Control) in duplicate should be used for calculating the mean and standard deviation. This is the $Negative_{mean}$.

The Cut-Off for Negative Samples is equal to a value greater than ($Negative_{mean}$ - 2*Standard Deviation).

Formula:
Negative Sample Value = OD > (*$Negative_{mean}$ *- 2*SD)

Typical example –

Sample Type	*Absorbance #1*	*Absorbance #2*	*Mean*
Negative	*2.561*	*2.928*	*2.744*

Therefore, Cut-Off = Mean - 2*SD
= 2.744 - (2*0.260)
= 2.744 - 0.519
= 2.225

Interpretation of Results:

Positive Samples *	**< Cut Off ***
Negative Samples *	**>= Cut Off ***
Unequivocal/Grey Zone Samples *	**<Cut Off - >(Cut Off-0.50)**

** The cutoff value is based on validation using recombinant antibodies in the assay. Users may set up their own cutoff values based on different patient serum panels from different geographic locations or ethnic backgrounds.*

Note:
1. If samples show false positive or false negative results; we recommend correlation with clinical interpretation.
2. If samples report unequivocal or grey zone absorbances, we recommend to re-test and report such samples as unequivocal samples, if clinical interpretation is not available.

Validity of the Test:

The use of controls allows validation of the test. The test is valid if the absorbance of Positive Control Value is lesser than 0.50. In case the control value is out of range, we recommend you to repeat the assay.

Quantitative Interpretation:
The Inhibition Rate of the Neutralizing Antibodies may also be measured. The user can determine the results of the sample by using the following formula:

$$\text{Inhibition} = 1 - \frac{\text{OD value of Sample}}{\text{OD value of Negative Control}} \times 100\%$$

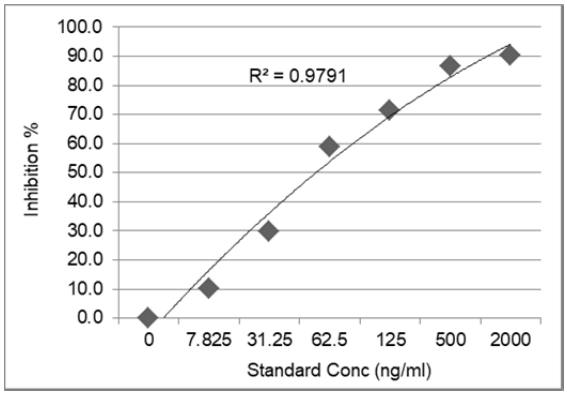
In the quantitative assay, determine the mean absorbance for each set of duplicate standards and samples. Plot the standard curve on standard graph paper, with neutralizing antibody concentration on the x-axis and inhibition rate % on the y-axis. Draw the best fit straight line through the standard points. To determine the unknowns neutralizing antibody concentrations, find the unknowns inhibition rate % value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the neutralizing antibody concentration.

Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 3rd order is best recommended for automated results.

Typical Data
(not to be used for your assay interpretation).

Standard Concentration (ng/ml)	Abs#1	Abs#2	Mean Abs	Inhibition %
0	2.194	1.889	2.042	0.0
7.825	2.000	1.663	1.832	10.3
31.25	1.495	1.370	1.433	29.8
62.5	0.934	0.743	0.838	58.9
125	0.555	0.610	0.582	71.5
500	0.275	0.275	0.275	86.5
2000	0.193	0.204	0.198	90.3

Typical Inhibition Graph



Limitation of the Procedure:
This ELISA test is designed for qualitative and/or quantitative detection of the neutralizing antibodies to SARS-CoV-2 only.

Performance Characteristics:

Specificity of the Neutralizing spike S1 Antibody (Standard, provided in the Kit):
The standard antibody used in the kit has cross-reactivity in ELISA with SARS-CoV-2 Epsilon (B.1.427, B.1.429). It has no cross-reactivity in ELISA SARS-CoV-2 Omicron (B.1.1.529) Spike S1 Protein, SARS-CoV-2 Omicron (B.1.1.529) Spike S1 Protein, MERS-CoV Spike S1 Protein, HCoV-HKU1 (isolate N1) Spike S1 Protein, HCoV-HKU1 (isolate N5) Spike S1 Protein, HCoV-NL63 Spike S1 Protein, HCoV-229E Spike S1 Protein, HCoV-OC43 Spike S1+S2 ECD Protein

Microneutralization (MN):
The neutralization activity is Measured by microneutralization assay in vitro. The virus microneutralizaiton (MN) test was performed on 293T-ACE2 cells infected with SARS-CoV-2 (2019-nCoV) Spike Pseudovirus under treatment of serial dilutions of neutralizing antibody. The infection was neutralized by increasing concentrations of Anti-SARS-CoV-2 Neutralizing Antibody. Rate of inhibition was determined by comparing the Relative Light Unit (RLU) of Luciferase reporter in different antibody concentrations. The IC50 is typically 0.11 ug/ml.

Characteristics of the spike S1 Protein:
The SARS-CoV-2 (2019-nCoV) Spike S1 Protein was expressed using a DNA sequence encoding the SARS-CoV-2 (2019-nCoV) Spike S1 (YP_009724390.1, with mutations W152C, L452R, D614G) (Met1-Arg685) was expressed. The mutations were identified in the SARS-CoV-2 variant (known as CAL.20C or B.1.429 lineage) which emerged in United States and the B.1.427/B.1.429 mutation was labeled as the Epsilon variant by WHO.

Safety Precautions:

- **This kit is For Research Use Only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

References:

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