GENLISA™ SARS-CoV-2 (Covid-19) Neutralizing Antibodies against spike mutation D614G ELISA

REF : KBVH500

Ver 1.0

RUO

Enzyme Immunoassay for the Qualitative Detection of all types of Neutralizing Antibodies against SARS-CoV-2 in a species- and isotype-independent manner in serum or plasma.

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 KBVH500 Σ 96 tests

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Introduction:

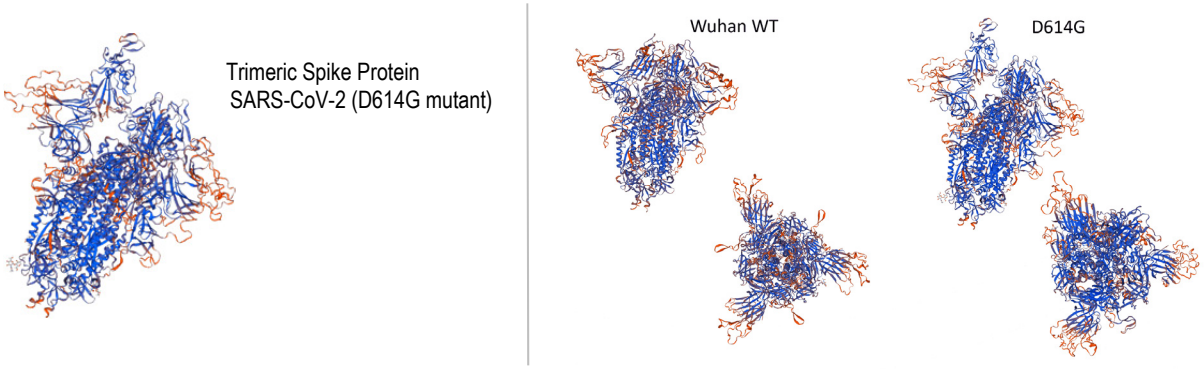
The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a blocking ELISA technique which mimics the virus neutralization process.

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).

Since the beginning of the COVID-19 pandemic in Wuhan, variants of the SARS-CoV-2 virus carrying a mutation in the spike protein at position 614 have largely replaced the original isolate. Established literatures have shown that the D614G mutation has a significant influence on the conformation of the trimeric spike. These structural changes in the G614 variant seem to enhance infectivity of virions through improved binding to the ACE2 receptor.

In addition, there are reports that antibodies generated against the Wuhan variant of SARS-CoV-2 may not fully protect against D614G.

We offer the Neutralizing Antibody Assay for the trimeric spike protein mutation D614G. This may help potential vaccine developers and others for comparative studies.



Intended Use:

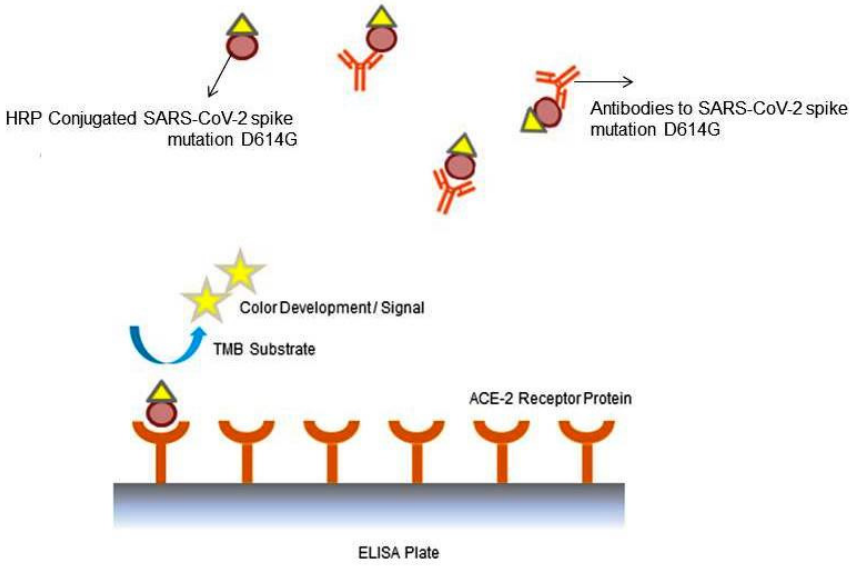
The GENLISA™ Neutralizing Antibodies against SARS-CoV-2 spike mutation D614G ELISA is used as an analytical tool for the qualitative detection all types of neutralizing antibodies against SARS-CoV-2 spike mutation D614G in serum or plasma.

Principle:

The method employs sandwich ELISA technique. The protein-protein interaction between HRP-D614G and hACE2 can be blocked by neutralizing antibodies against D614G.

Samples and controls are pipetted in a blank microtitre plate and incubated with HRP conjugated human SARS-CoV-2 spike mutation D614G protein. The antibodies to SARS-CoV-2 spike mutation D614G present in the samples and controls bind to the SARS-CoV-2 spike mutation D614G protein to form a complex.

This solution of bound and unbound antibodies to SARS-CoV-2 spike mutation D614G is then pipetted into human ACE2 coated microplate. After washing to remove the unbound complex and proteins, the substrate solution (TMB) is added to the microwells. Post incubation, color develops proportionally to the amount of Antibodies to SARS-CoV-2 (Covid-19) spike mutation D614G present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

1. Blank Microtitre plate (12 x 96 wells) - 1 no.
2. Human ACE-2 Coated Microtiter Plate (12 x 8 wells) - 1 no
3. Positive Control (0.5 ml/vial) - 1 vial
4. Negative Control (0.5 ml/vial) - 1 vial
5. SARS-CoV-2 spike mutation D614G:HRP Conjugate -12 ml
6. (5X) Sample Diluent - 50 ml
7. (20X) Wash Buffer - 25 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml

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 2-8°C.
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:

1. 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 1:1000 (v/v) for optimal recovery, (for example 1 ul sample + 999 ul assay diluent) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted less or more respectively with Assay 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 Assay 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. To make **Assay Diluent (1X)**; dilute **50 ml** of **5X Assay Diluent** in **200 ml of DI water**.
4. To make **Wash Buffer (1X)**; dilute **25 ml** of **20X Wash Buffer** in **475 ml of DI water**.

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 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 Samples.

Assay Procedure:

A) Neutralization Reaction

1. Pipette **100 ul** of **Negative Control** in duplicate to the respective wells in the blank microplate.

2. Pipette **100 ul** of **Positive Control** in duplicate to the respective wells in the blank microplate.

3. Add **100 ul** of **SARS-CoV-2 mutation D614G:HRP Conjugate** into all the wells.

4. 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** 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.

Interpretation of Results:

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	*0.561*	*0.528*	*0.545*
Standard Deviation	*0.561-0.545*	*0.528-0.545*	
	= 0.016	*= 0.017*	

Mean Standard Deviation = $\sqrt{(0.016)^2 + (0.017)^2 / 2}$ *= 0.0234*

Therefore Cut-Off = Mean + 2*SD
= 0.545 + (2*0.0234)
= 0.545 + 0.0468
= 0.592

** 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 different ethnic backgrounds.*

Conclusion:

Positive Samples *	**< Cut Off ***
Negative Samples *	**>= Cut Off ***
Positive Control Value	**< 0.80**

Limitation of the Procedure:

- This ELISA test is designed for qualitative detection of the neutralizing antibodies only.

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:

Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses
S Jiang, C Hillyer, L Du - Trends in immunology, 2020 - Elsevier

Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein
AC Walls, YJ Park, MA Tortorici, A Wall, AT McGuire… - Cell, 2020 - Elsevier

A human monoclonal antibody blocking SARS-CoV-2 infection
C Wang, W Li, D Drabek, NMA Okba… - Nature …, 2020 - nature.com

SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor
M Hoffmann, H Kleine-Weber, S Schroeder, N Krüger… - Cell, 2020 - Elsevier

Potent human neutralizing antibodies elicited by SARS-CoV-2 infection
B Ju, Q Zhang, X Ge, R Wang, J Yu, S Shan, B Zhou… - BioRxiv, 2020 - biorxiv.org

Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor
J Lan, J Ge, J Yu, S Shan, H Zhou, S Fan, Q Zhang… - Nature, 2020 - nature.com

A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV
M Yuan, NC Wu, X Zhu, CCD Lee, RTY So, H Lv… - …, 2020 - science.sciencemag.org

Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV
X Ou, Y Liu, X Lei, P Li, D Mi, L Ren, L Guo… - Nature …, 2020 - nature.com

Duration of serum neutralizing antibodies for SARS-CoV-2: lessons from SARS-CoV infection
Q Lin, L Zhu, Z Ni, H Meng, L You - Journal of Microbiology …, 2020 - ncbi.nlm.nih.gov

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