GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgG spike protein Qualitative ELISA

REF : KBVH015-2

Ver 3.0

RUO

This Kit has been Calibrated against an International Standard from the National Institute of Biologicals and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK.

Enzyme Immunoassay for the Qualitative estimation of IgG Antibodies to Human SARS-CoV-2 (Covid-19) in human serum and plasma and cell culture supernatant

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 KBVH015-2 **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 sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

There is no standard reference SARS-CoV-2 antigen material available; accordingly, absolute analytical sensitivity cannot be calculated. The kit uses a polyclonal antibody as Positive Control. The assay allows the qualitative determination of samples.

Intended Use:

The GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgG ELISA kit is used as an analytical tool for qualitative estimation of IgG antibodies to Human SARS-CoV-2 (Covid-19) in human serum, plasma and cell culture supernatant.

Principle:

The method employs sandwich ELISA technique. Human SARS-CoV-2 protein is pre-coated onto microwells. Samples and controls are pipetted into microwells and IgG Antibodies to human SARS-CoV-2 (Covid-19) present in the sample are bound by the protein antigen. After incubation the wells are washed and followed by HRP-conjugated Detection IgG Antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to the microwells. Color develops proportionally to the amount of IgG Anti-Human SARS-CoV-2 (Covid-19) in the samples. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Recombinant SARS-CoV-2 (Covid-19) spike protein Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with SARS-CoV-2 (Covid-19) spike protein	1 x 96 wells
Positive Control	Lyophilized Anti-Human SARS-CoV-2 (Covid-19) spike protein IgG Standard.	2 vials
Goat Anti-Human IgG:HRP Conjugate	Goat Anti-Human IgG: HRP Conjugate prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(5X) Sample Diluent	Buffered protein base with preservative thiomersol < 0.01%	2 x 50 ml
(1X) Control Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane with 1:5000 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

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.

Serum and Plasma samples should be diluted 1:5000 (v/v) for optimal recovery, (for example 1 ul sample + 4999 ul (1X) Sample diluent) prior to assay. ***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*

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or lower temperature. Avoid repeated freeze-thaw cycles. If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.

Should you desire to inactivate the virus, use a (5X) Lysis Buffer (optional, not provided). Add 1/5 volume of (5X) Lysis Buffer to sample (i.e. add 50 ul (5X) Lysis Buffer to 200 ul sample). Vortex well.

Note:
The sample should be diluted to within the working range of the assay in (1X) Sample Diluent. The exact dilution must be determined based on the concentration of specific target in individual samples.

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 **(1X) Wash Buffer**; dilute **25 ml of 20X Wash Buffer in 380 ml of DI water**.
4. To make **(1X) Sample Diluent**; dilute **50 ml** of **(5X) Sample Diluent** in **200 ml of DI water**.
5. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of Control Diluent to obtain Positive Control. Keep the vial for 15 mins with gentle agitation and then run the assay procedure. Use the Control 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 erroneous results for the presence of Anti-Human SARS-CoV-2 (Covid-19).
3. It is recommended that the Controls 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 Controls and Samples.

Assay Procedure:

1. Pipette **100 ul** of **Controls** and **diluted Samples** to the respective wells. Seal plate and incubate for 1 hour at Room Temperature (18-25ºC).

2. 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.

3. Add **100 ul** of **Anti-Human IgG:HRP Conjugate** to each well.

4. Seal plate and incubate for 1 hour at Room Temperature (18-25°C).

5. Wash plate 4 times with **Wash Buffer (1X)** as in step 2.

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

7. Incubate in the dark for 15 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.

Calculation for Cut off Values

Read the sample and negative 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 Positives is equal to a value greater than ($Negative_{mean}$ + 3*Standard Deviation).

Formula:
Positive Sample Value = OD > (*Negative$_{mean}$ *+ 3*SD)

Typical example –

Sample Type	*Absorbance #1*	*Absorbance #2*	*Mean*
Negative	*0.131*	*0.128*	*0.129*

Therefore Cut-off = Mean + 3*SD *(Classen et al. 1987)*
= 0.129 + 3* 0.0021
= 0.129 + 0.0042
= 0.133

** 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.*

Interpretation of Results:

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

Note:
1. Incase your samples show false positive or false negative results, we recommend to increase the cut-off to 5*SD. (*Classen et al. 1987*).
2. Incase your samples report absorbance close to the cut-off absorbance, we recommend to report such samples as unequivocal samples in absence of clinical interpretation.

Validity of the Test:
The use of controls allows validation of the test. The test is valid if the following conditions are met,

Positive Control Value	**> Cut Off**
Negative Control Value	**Absorbance < 0.5**

In case the control value is out of range, we recommend you to repeat the assay.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct. The test is only for screening purpose only and the results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical symptoms in conjunction to other tests.

In the first week of the onset of the infection with the novel coronavirus (COVID-19) patients results may be negative for IgG. In addition, patients with low imm8unity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgG. Patients previously infected with SARS or other coronavirus strain may cause a light IgG positive in view of similarity of different strains.

Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.

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.

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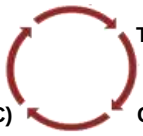
SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

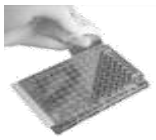
Components (2-8ºC) → 30 mins RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) →  → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

3.  Pipette **100 ul Controls and diluted Samples** into the respective wells.

4. Cover plate  and **incubate** for 

at Room Temperature.

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

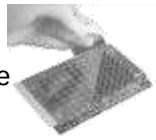
6.  Pipette **100 ul Anti-Human IgG:HRP Conjugate** into each well.

7. Cover plate  and **incubate** for 

at Room Temperature.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and **incubate** for 

at Room Temperature.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Results
1A 2A	+ve Control +ve Control			
1B 2B	-ve Control -ve Control			
1C 2C	*Sample*			
1D 2D	*Sample*			
1E 2E	*Sample*			
1F 2F	*Sample*			
1G 2G	*Sample*			
1H 2H	*Sample*			
3A 4A	*Sample*			
3B 4B	*Sample*			

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