GENLISA™ Human SARS-CoV-2 (Covid-19) Spike Protein Antigen Qualitative ELISA

REF : KBVH015-4

Ver 1.2

RUO

Enzyme Immunoassay for the Qualitative Antigen Determination of SARS-CoV-2 (Covid-19) Spike Protein 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 KBVH015-4 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.

Intended Use:

The GENLISA™ Human SARS-CoV-2 (Covid-19) Spike Protein Antigen ELISA kit is used as an analytical tool for qualitative antigen determination of Human SARS-CoV-2 (Covid-19) spike proteins in human serum and plasma and cell culture supernatant.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Human SARS-CoV-2 (Covid-19) spike proteins present in the sample are bound by the capture antibodies. After incubation the wells are washed and followed by HRP- conjugated Detection 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 microwells and color develops proportionally to the amount of Human SARS-CoV-2 (Covid-19) spike proteins in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. SARS-CoV-2 (Covid-19) spike protein Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Human SARS-CoV-2 spike protein Positive Control (concentrated, lyophilized) - 2 vials
3. SARS-CoV-2 (Covid-19) Antibody:HRP Conjugate - 12 ml
4. (5X) Assay Diluent - 50 ml
5. Control Diluent - 10 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual
10. Plate Sealer

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 1:100 (v/v) for optimal recovery, (for example 1 ul sample + 99 ul (1X) Assay Diluent) prior to assay. 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*

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.

Note:
The sample should be diluted to within the working range of the assay in 1X Assay 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 **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**.
5. **Positive Control**: Reconstitute the concentrated Positive Control lyophilized vial with 1 ml of Control Diluent. Keep the vial for 15 mins with gentle agitation before using. Use Control Diluent as **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 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 **reconstituted Positive Control, Negative Control** and **diluted Samples** to the respective wells.

2. Seal plate and incubate for 1 hour at 37ºC shaking at 180 rpm.

3. 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.

4. Add **100 ul** of **SARS-CoV-2 Antibody:HRP Conjugate** to each well.

5. Seal plate and incubate for 1 hour at 37°C shaking at 180 rpm.

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

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

8. Incubate in the dark for 30 minutes at Room Temperature.

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

10. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Interpretation of Results:

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*
Standard Deviation	*0.131-0.129*	*0.128-0.129*	
	= 0.002	*= -0.001*	

Mean Standard Deviation = $\sqrt{(0.002)^2 + (-0.001)^2 / 2} = 0.0014$

Therefore Cut-off = Mean + 3*SD
= 0.129 + 3* 0.0014
= 0.129 + 0.0042
= 0.133

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

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.

Performance Characteristics of the Kit:

This kit has been validated. Please view the details herein below.

Sensitivity:
The capture antibodies used in the kit are specific against 2019-nCoV Coronavirus spike and has 100% cross-reactivity with SARS-CoV Spike S1 Protein, SARS-CoV Spike RBD Protein.

A DNA sequence encoding the SARS-CoV-2 (2019-nCoV) Spike Protein (S1 Subunit) (YP_009724390.1) (Met1-Arg685) was expressed in baculovirus-insect cells and is used as control in the kit.

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.

SCHEMATIC ASSAY PROCEDURE

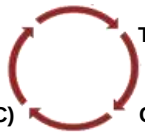
1. Remove all components, 30 minutes before adding into the assay plate.

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

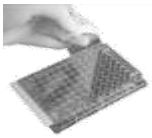
→ Thaw at Room Temperature (18-24ºC) → USE NOW

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) → AVOID → NO CHANGE IN RESULTS



3.  Pipette **100 ul** reconstituted **Positive Control, Negative Control** and **diluted Samples** into the respective wells.

4. Cover plate  and **incubate** for 

at 37 ºC shaking at 180 rpm.

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

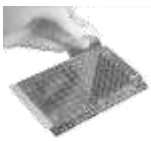
6.  Pipette **100 ul SARS-CoV-2 Antibody:HRP Conjugate** into each well.

7. Cover plate  and **incubate** for 

at 37 ºC shaking at 180 rpm.

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

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	Positive Control			
2A	Positive Control			
1B	Negative Control			
2B	Negative Control			
1C	*Sample*			
2C	*Sample*			
1D	*Sample*			
2D	*Sample*			
1E	*Sample*			
2E	*Sample*			
1F	*Sample*			
2F	*Sample*			
1G	*Sample*			
2G	*Sample*			
1H	*Sample*			
2H	*Sample*			

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