GENLISA™ Human SARS-CoV-2 (Covid-19) Spike Protein S1 Antigen Quantitative ELISA

REF : KBVH015-10

Ver 2.2

RUO

Enzyme Immunoassay for the Quantitative 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 **KBVH015-10** **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 S1 Antigen ELISA kit is used as an analytical tool for quantitative 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 standards 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 S1 Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Human SARS-CoV-2 spike protein S1 Standards (concentrated, lyophilized 5ug/ml) - 2 vials
3. SARS-CoV-2 (Covid-19) Antibody:HRP Conjugate - 12 ml
4. (5X) Assay Diluent - 50 ml
5. Standard 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. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent to obtain a concentration of 5ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 400 ul of original **Standard (5 ug/ml)** with 100 ul of Standard Diluent to generate a **4000 ng/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
5 ug/ml	Original Standard	Original Standard provided in the Kit
4000 ng/ml	Standard No.7	400 ul Original Standard (5 ug/ml) + 100 ul Standard Diluent
2000 ng/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard Diluent
1000 ng/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent
500 ng/ml	Standard No.4	250 ul Standard No.5 + 250 ul Standard Diluent
250 ng/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard Diluent
125 ng/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard Diluent
62.5 ng/ml	Standard No.1	250 ul Standard No.2 + 250 ul Standard Diluent
0 ng/ml	Standard No. 0	Only Standard Diluent

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 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:

1. Pipette **100 ul** of **Standards** 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.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human SARS-CoV-2 (Covid-19) spike protein concentrations, find the unknown's Mean Absorbance 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 Human SARS-CoV-2 (Covid-19) spike protein Concentration.

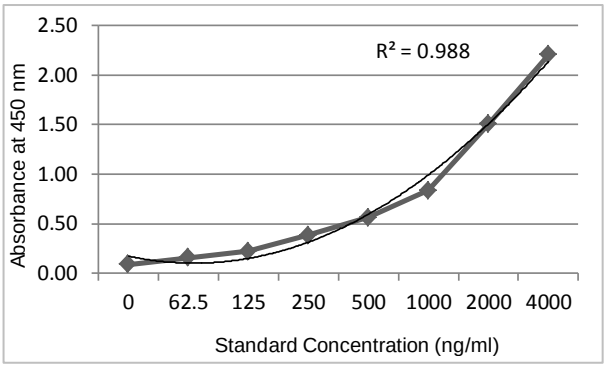
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or a polynomial 2nd order curve is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Typical Data

Standards provided (ng/ml)	Absorbance 1	Absorbance 2	Mean Absorbance
0	0.093	0.084	0.089
62.5	0.16	0.155	0.158
125	0.216	0.224	0.220
250	0.387	0.384	0.386
500	0.551	0.577	0.564
1000	0.88	0.798	0.839
2000	1.521	1.494	1.508
4000	2.21	2.207	2.209

Typical Graph



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.

Standard Calibration Range:
0 ng/ml - 4000 ng/ml

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 calibrator in the kit.

Limit Of Detection:
It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD.
10 replicates of '0' standards were evaluated and the LOD was found to be less than 60 ng/ml.

Precision:
Intra-Assay
Three samples of known concentration were tested five times on one plate to assess intra-assay precision.
Inter-Assay: Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

	Intra-Assay			Inter-Assay		
Sample	1	2	3	1	2	3
N	5	5	5	5	5	5
Mean (ng/ml)	1586	980	457	1590	1293	1725
CV (%)	14.2 %	10.0 %	9.5 %	10.0 %	12.7 %	9.5 %

Spike and Recovery:
SARS-CoV-2 (Covid-19) spike proteins was spiked at different levels to measure mean recovery.

Sample	Mean % Recovery	Range
Serum (n=3)	80.4	78-84 %
Cell Culture Supernates (n=3)	114	105-125 %

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.

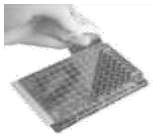
Components (2-8ºC) → 30 mins RT Before Use → 

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

3.  Pipette **100 ul Standards** 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 2A	0 Standard 0 Standard			
1B 2B	62.5 ng/ml 62.5 ng/ml			
1C 2C	125 ng/ml 125 ng/ml			
1D 2D	250 ng/ml 250 ng/ml			
1E 2E	500 ng/ml 500 ng/ml			
1F 2F	1000 ng/ml 1000 ng/ml			
1G 2G	2000 ng/ml 2000 ng/ml			
1H 2H	4000 ng/ml 4000 ng/ml			
3A 4A	*Sample*			
3B 4B	*Sample*			

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