GENLISA™ Human SARS-CoV-2 (Covid-19) Envelope Protein Antigen Quantitative TITRATION ELISA

REF : KBVH015-30

Ver 1.2

RUO

Enzyme Immunoassay for the Quantitative Antigen Determination of SARS-CoV-2 (Covid-19) Envelope Protein in human serum and 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 KBVH015-30 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 competitive ELISA technique.

Intended Use:

The GENLISA™ Human SARS-CoV-2 (Covid-19) Envelope Protein Antigen ELISA kit is used as an analytical tool for quantitative antigen determination of Human SARS-CoV-2 (Covid-19) Envelope Proteins in human serum and plasma.

Principle:

The method employs competitive ELISA technique. Samples and standards pipetted into microwells which are pre-coated with SARS-CoV-2 Envelope Protein antibody. SARS-CoV-2 Envelope Protein HRP:Conjugate is added which competes with the sample 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) Envelope Proteins in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm. The concentration of the SARS-CoV-2 Envelope Protein in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Materials Provided:

Part	Description	Qty
SARS-CoV-2 (Covid-19) Envelope Protein Antibody Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with SARS-CoV-2 (Covid-19) Envelope Protein Antibody	1 x 96 wells
Human SARS-CoV-2 Envelope Protein Standard	Lyophilized Human SARS-CoV-2 Envelope Protein Standards Concentration – 5000 ng/ml	2 vials
SARS-CoV-2 (Covid-19) Envelope Protein:HRP Conjugate	SARS-CoV-2 (Covid-19) Envelope Protein: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 protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	2 x 50 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane 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

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 TMB 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 1:1000 (v/v) for optimal recovery, (for example 1 ul sample + 999 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.
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Note: *Grossly hemolyzed samples are not suitable for use in this assay*

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 **Wash Buffer (1X)**; dilute **25 ml** of **20X Wash Buffer** in **475 ml of DI water**.
4. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent to obtain a concentration of 5000 ng/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 300 ul of original **Standard (5000 ng/ml)** with 200 ul of Standard Diluent to generate a **3000 ng/ml Standard Solution**. Prepare further **Standards** by 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
5000 ng/ml	Original Standard	Original Standard provided in the Kit + 1 ml of Standard Diluent
3000 ng/ml	Standard No.6	300 ul Original Standard (5000 ng/ml) + 200 ul Standard Diluent
2000 ng/ml	Standard No.5	200 ul Original Standard (5000 ng/ml) + 300 ul Standard Diluent
1500 ng/ml	Standard No.4	250 ul Standard No.6 + 250 ul Standard Diluent
1000 ng/ml	Standard No.3	250 ul Standard No.5 + 250 ul Standard Diluent
250 ng/ml	Standard No.2	125 ul Standard No.3 + 375 ul Standard Diluent
62.5 ng/ml	Standard No.1	125 ul Standard No.2 + 375 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. Add **100 ul** of **SARS-CoV-2 Protein:HRP Conjugate** to each well.

3. Seal plate and incubate for 90 mins at room temperature.

4. 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.

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

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

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

8. 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) Envelope 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) Envelope 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 2^{nd} order curve is best recommended for automated results.

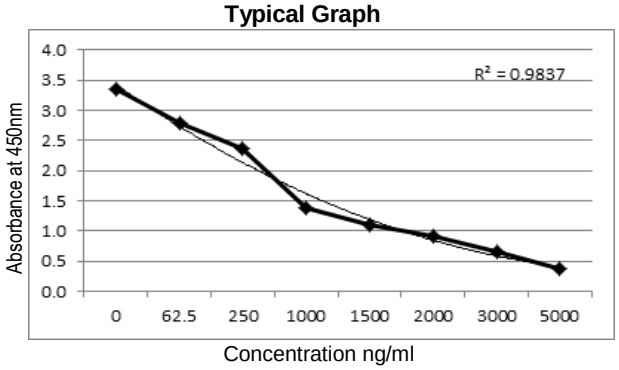
Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Typical Data

Standards (ng/ml)	Abs 1	Abs 2	Mean Abs	Interpolated conc.
0	3.348	3.344	3.346	--
62.5	2.909	2.692	2.800	76.0
250	2.490	2.229	2.360	218.8
1000	1.455	1.312	1.383	1031.7
1500	1.204	1.020	1.112	1520.2

2000	0.963	0.873	0.918	2018.6
3000	0.714	0.601	0.657	3016.1
5000	0.387	0.365	0.376	4872.5

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 - 5000 ng/ml

Sensitivity:
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 ~58 ng/ml.

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<15%	<15%
Medium	<12%	<12%
High	<12%	<12%

Recovery:
Human sera was measured with two replicates and two runs (n = 5). The Human sera were pooled patient and single donor spiked samples. Samples were measured using one lot of reagent. All data met our acceptance criteria for % CV and 95% (CI) Confidence Intervals for % CV.

Matrix	Recovery Range %
Serum (n=5)	80 - 120
Plasma (n=5)	80 - 120

Note: Serum was diluted using Sample Diluent provided with 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) → COOL (2-8ºC) → THAW (22±4ºC) → AVOID → NO CHANGE IN RESULTS

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

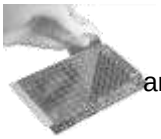
4.  Pipette **100 ul SARS-CoV-2 Protein:HRP Conjugate** into each well.

5. Cover plate  and **incubate** for 

at room temperature.

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

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

8 Cover plate  and **incubate** for 

at Room Temperature.

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

10. 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	250 ng/ml 250 ng/ml			
1D 2D	1000 ng/ml 1000 ng/ml			
1E 2E	1500 ng/ml 1500 ng/ml			
1F 2F	2000 ng/ml 2000 ng/ml			
1G 2G	3000 ng/ml 3000 ng/ml			
1H 2H	5000 ng/ml 5000 ng/ml			
3A 4A	*Sample*			
3B 4B	*Sample*			

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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Recombinant SARS-CoV-2 envelope protein antibody Coated Microtiter Plate
STD	Human SARS-CoV-2 Envelope Protein Standards
HRP CONJ	Anti SARS-CoV-2:HRP Conjugate
STD DIL	(1X) Standard Diluent
1X SAMP DIL	(1X) Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature