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

REF : KBVH015-31

Ver 1.1

RUO

Enzyme Immunoassay for the Quantitative Antigen Determination of SARS-CoV-2 (Covid-19) Membrane 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 **KBVH015-31** **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) Membrane Protein Antigen ELISA kit is used as an analytical tool for quantitative antigen determination of Human SARS-CoV-2 (Covid-19) Membrane Proteins in human serum and plasma.

Principle:

The method employs sandwich ELISA technique. Standards or Samples are added to the microtiter well which is pre-coated with SARS-CoV-2 Membrane Protein. Plates are washed with wash buffer to remove unbound sample. Anti- Human IgG HRP Conjugate is added to the microplate and incubated to form a complex. After incubation and a washing step TMB Substrate is added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the SARS-CoV2 Membrane Protein in the samples is directly proportional to the yellow color developed (absorbance) in the wells. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Recombinant SARS-CoV-2 (Covid-19) Membrane Protein Antibody Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with SARS-CoV-2 (Covid-19) Membrane Protein Antibody	1 x 96 wells
Human SARS-CoV-2 Membrane Protein Standard	Lyophilized Human SARS-CoV-2 Membrane Protein Standards Concentration – lyophilized; 2500 ng/ml	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
(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 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 Sample Diluent (1X) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted with Sample Diluent (1X) 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*

Note:
The sample should be diluted to within the working range of the assay in Sample Diluent (1X). 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 0.5 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 400 ul of original Standard (5000 ng/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
2500 ng/ml	Lyophilized Standard	Lyophilized Standard provided in the Kit
5000 ng/ml	Reconstituted Standard	Lyophilized standard + 0.5 ml Standard Diluent (1X)
4000 ng/ml	Standard No. 8	400 ul Reconstituted Standard + 100 ul Standard Diluent
2000 ng/ml	Standard No.7	250 ul Standard No.8 + 250 ul Standard Diluent
1000 ng/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard Diluent
500 ng/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent
250 ng/ml	Standard No.4	333.4 ul Standard No.5 + 166.6 ul Standard Diluent
125 ng/ml	Standard No.3	250 ul Standard No.4 + 250 ul Standard Diluent
31.25 ng/ml	Standard No.1	250 ul Standard No.2 + 250 ul Standard Diluent
0 ng/ml	Standard No. 0	Only Standard Diluent

Use the Standards as soon as possible upon reconstitution. Discard balance standard after use.

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 **Goat Anti-Human IgG: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) Membrane 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) Membrane Protein Concentration.

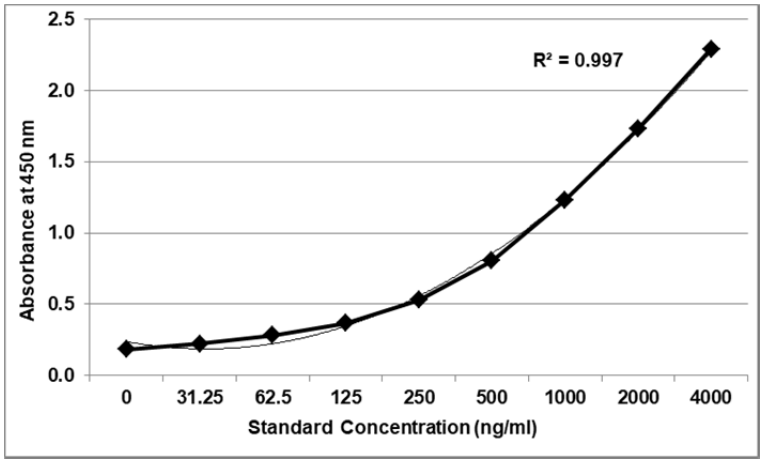
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or a polynomial 2^{nd} order curve is best recommended for automated results.

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

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.182	0.186	0.184	3.1	--
31.25	0.231	0.217	0.224	27.3	87.3
62.5	0.278	0.286	0.282	64.8	103.8
125	0.379	0.353	0.366	123.0	98.4
250	0.519	0.538	0.528	247.0	98.8
500	0.782	0.827	0.804	498.2	99.6
1000	1.229	1.238	1.233	1021.9	102.2
2000	1.699	1.759	1.729	1965.5	98.3
4000	2.322	2.261	2.291	4023.2	100.6

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.

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

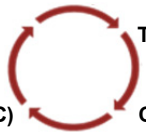
1. Remove all components, 30 minutes before adding into the assay plate.

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

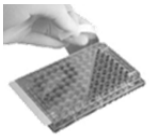
Thaw at Room Temperature (18-24ºC) → USE NOW

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

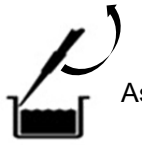
Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → AVOID → NO CHANGE IN RESULTS



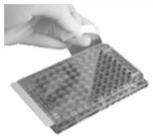
3.  Pipette **100 ul Standards** and **diluted Samples** into the respective wells.

4. Cover plate  and **incubate** for 

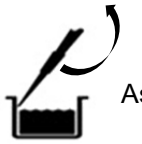
at 37 ºC shaking at 180 rpm.

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

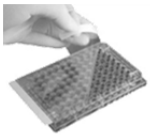
6.  Pipette **100 ul Goat Anti-Human IgG:HRP Conjugate** into each well.

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	31.25 ng/ml 31.25 ng/ml			
1C 2C	62.5 ng/ml 62.5 ng/ml			
1D 2D	125 ng/ml 125 ng/ml			
1E 2E	250 ng/ml 250 ng/ml			
1F 2F	500 ng/ml 500 ng/ml			
1G 2G	1000 ng/ml 1000 ng/ml			
1H 2H	2000 ng/ml 2000 ng/ml			
3A 4A	4000 ng/ml 4000 ng/ml			
3B 4B	*Sample*			

LIMITED WARRANTY

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This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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