GENLISA™ Human SARS-CoV-2 (2019-nCoV) Spike RBD Antigen Quantitative ELISA

REF : KBVH015-12

Ver 1.2

RUO

Enzyme Immunoassay for the Quantitative Estimation of SARS-CoV-2 (2019-nCoV) Spike RBD Antigen 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 KBVH015-12 **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.

The spike (S) glycoprotein of coronaviruses contains protrusions that will only bind to certain receptors on the host cell. Known receptors which bind S1 are ACE2 (angiotensin-converting enzyme 2), DPP4 (dipeptidyl peptidase-4), APN (aminopeptidase N), CEACAM (carcinoembryonic antigen-related cell adhesion molecule 1), Sia (sialic acid), O-ac Sia (O-acetylated sialic acid). The spike is essential for both host specificity and viral infectivity.

The term 'peplomer' is typically used to refer to a grouping of heterologous proteins on the virus surface that function together. The spike (S) glycoprotein of coronaviruses is known to be essential in the binding of the virus to the host cell at the advent of the infection process. It's been reported that 2019-nCoV can infect the human respiratory epithelial cells through interaction with the human ACE2 receptor.

The spike protein is a large type I transmembrane protein containing two subunits, S1 and S2. S1 mainly contains a receptor binding domain (RBD), which is responsible for recognizing the cell surface receptor. S2 contains basic elements needed for the membrane fusion. The S protein plays key parts in the induction of neutralizing-antibody and T-cell responses, as well as protective immunity.

The main functions for the Spike protein are summarized as:

- Mediate receptor binding and membrane fusion;
- Defines the range of the hosts and specificity of the virus;
- Main component to bind with the neutralizing antibody;
- Key target for vaccine design;
- Can be transmitted between different hosts through gene recombination or mutation of the receptor binding domain (RBD), leading to a higher mortality rate.

Intended Use:

The GENLISA™ Human SARS-CoV-2 (2019-nCoV) Spike RBD Quantitative ELISA kit is used as an analytical tool for quantitative estimation of SARS-CoV-2 (2019-nCoV) Spike RBD Antigen in human serum.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibody specific for SARS-CoV-2 (2019-nCoV) Spike RBD is pre-coated onto microwells. Samples and standards are pipetted into microwells and SARS-CoV-2 (2019-nCoV) Spike RBD Antigen present in the sample is bound by the immobilized antibody. After incubation the wells are washed and followed by addition of HRP-conjugated Detection anti- SARS-CoV-2 (2019-nCoV) Spike RBD antibody into each well 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 SARS-CoV-2 (2019-nCoV) Spike RBD in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Human Anti-SARS-CoV-2 (2019-nCoV) Spike RBD Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Human Anti-SARS-CoV-2 (2019-nCoV) Spike RBD antibody	1 x 96 wells
Human SARS-CoV-2 (2019-nCoV) Spike RBD Standard	Lyophilized Human SARS-CoV-2 (2019-nCoV) Spike RBD Standard Concentration – 1000 ng/ml	2 vials
Human Anti-SARS-CoV-2 (2019-nCoV) Spike RBD antibody: HRP Conjugate	Human Anti-SARS-CoV-2 (2019-nCoV) Spike RBD antibody: 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.	20 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane	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. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

Samples should be diluted 1:10 (v/v) for optimal recovery, (for example 1 ul sample + 9 ul 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*

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 1000 ng/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 360 ul of original **Standard (1000 ng/ml)** with 140 ul of Standard Diluent to generate a **720 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
1000 ng/ml	Original Standard	Original Standard provided in the Kit
720 ng/ml	Standard No.7	360 ul Original Standard (1000 ng/ml) + 140 ul Standard Diluent
360 ng/ml	Standard No.6	250 ul Standard No.7 + 250 ul Standard Diluent
180 ng/ml	Standard No.5	250 ul Standard No.6 + 250 ul Standard Diluent
90 ng/ml	Standard No.4	250 ul Standard No.5 + 250 ul Standard Diluent
60 ng/ml	Standard No.3	333.4 ul Standard No.4 + 166.6 ul Standard Diluent
30 ng/ml	Standard No.2	250 ul Standard No.3 + 250 ul Standard Diluent
15 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 under-estimation of the amount of SARS-CoV-2 (2019-nCoV).
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 the 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 **Human Anti-SARS-CoV-2 (2019-nCoV): HRP Conjugate** to each well.

5. Seal the 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 3.
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 standard 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 SARS-CoV-2 Spike RBD Antigen 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 concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a polynomial regression (2^{nd} order) or a cubic spline curve-fit 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.

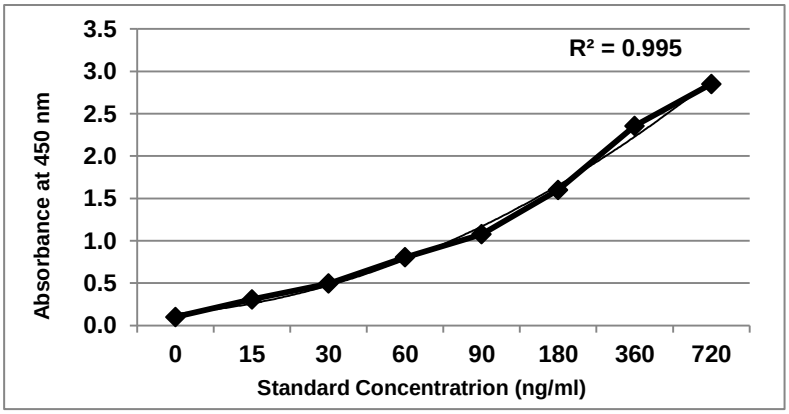
Quality Control:

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.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.100	0.098	0.099	--	--
15	0.304	0.311	0.307	15.4	103.0
30	0.489	0.499	0.494	31.0	103.3
60	0.794	0.814	0.804	60.3	100.5
90	1.084	1.066	1.075	90.9	101.0
180	1.717	1.483	1.600	170.3	94.6
360	2.400	2.310	2.355	381.9	106.1
720	2.921	2.779	2.850	700.9	97.3

Typical Graph



Performance Characteristics of the Kit:

Sensitivity:
Limit Of Detection: There is no standard reference SARS-CoV-2 spike RBD material available; accordingly, absolute analytical sensitivity cannot be calculated. Based on the kit working standards the LOD is 12 ng/ml

Specificity:
Reactivity/Inclusivity.
Mutations in the SARS-CoV-2 genome have been identified as the virus has spread, but no serologically unique strains have been described relative to the originally isolated virus (this research is limited at present).

Traceability:
There are no reference standards for SARS-Cov-2 RBD Antigen. The results are reported in dilution titres and the method has been standardized in our laboratory at KRISHGEN BIOSYSTEMS.

Linearity:
Standards provided in the kit were used for measuring the linearity range of SARS-CoV-2 RBD Antigen present in serum and plasma.

Human Serum Dilution	Standards provided (ng/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
Human Serum 1:10 dilution using Sample Diluent	0	0.103	--	--
	720	2.971	837.1	116.3
Human Serum 1:100 dilution using Sample diluent	0	0.202	7.2	--
	720	2.990	862.2	119.8
Human Serum 1:500 dilution using Sample diluent	0	0.086	--	--
	720	2.782	638.7	88.7
Human Serum 1:1000 dilution using Sample diluent	0	0.098	--	--
	720	2.768	626.9	87.1
Human Serum 1:2000 dilution using Sample diluent	0	0.099	--	--
	720	2.968	833.2	115.7
Human Serum 1:5000 dilution using Sample diluent	0	0.091	--	--
	720	2.962	825.6	114.7

Human Plasma Dilution	Standards provided (ng/ml)	Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
Human Plasma 1:10 dilution using Sample diluent	0	0.116	0.6	--
	720	2.850	700.9	97.3
Human Plasma 1:100 dilution using Sample diluent	0	0.212	8.0	--
	720	2.944	803.3	111.6
Human Plasma 1:500 dilution using Sample diluent	0	0.099	--	--
	720	2.718	587.3	81.6
Human Plasma 1:1000 dilution using Sample diluent	0	0.090	--	--
	720	2.625	522.6	72.6
Human Plasma 1:2000 dilution using Sample diluent	0	0.100	--	--
	720	2.911	764.9	106.2
Human Plasma 1:5000 dilution using Sample diluent	0	0.069	--	--
	720	2.857	707.8	98.3

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	<12%	<12%
Medium	<10%	<10%
High	<10%	<10%

Recovery:

Sera and plasma was measured with two replicates and two runs (n = 5). The sera and plasma 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 and Plasma were 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 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.

References:

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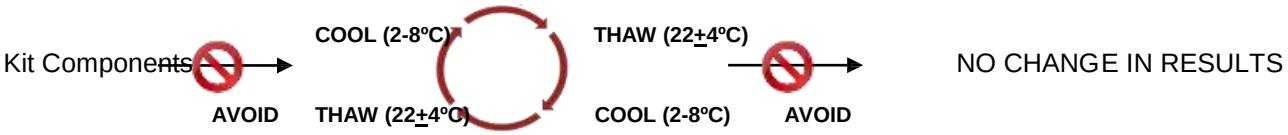
SCHEMATIC ASSAY PROCEDURE

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

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



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

4. Cover plate and **incubate** for 60 min 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 60 min 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 30 min at Room Temperature.

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

12. Read absorbance at 450nm with a microplate reader within 30 min 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	15 ng/ml 15 ng/ml			
1C 2C	30 ng/ml 30 ng/ml			
1D 2D	60 ng/ml 60 ng/ml			
1E 2E	90 ng/ml 90 ng/ml			
1F 2F	180 ng/ml 180 ng/ml			
1G 2G	360 ng/ml 360 ng/ml			
1H 2H	720 ng/ml 720 ng/ml			
3A 4A	*Sample*			
3B 4B	*Sample*			

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components notmanufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components.

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THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be torepair 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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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Human Anti-SARS-CoV-2 Spike RBD Coated Microtiter Plate
STD	Human SARS-CoV-2 (2019-nCoV) Spike RBD Standard
HRP CONJ	Human Anti-SARS-CoV-2 Antibody:HRP Conjugate
1X 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