GENLISA™ Human Anti-Coronavirus IgG ELISA

REF: KBVH015-1

Ver 3.1

RUO

Enzyme Immunoassay for the Quantitative determination of Human SARS-CoV IgG (Coronavirus) in human serum and plasma, respiratory specimens 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 KBVH015-1 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.

Intended Use:

The GENLISA™ Human Anti-Coronavirus IgG ELISA kit is used as an analytical tool for quantitative determination of IgG against coronavirus in human serum and plasma, respiratory specimens and cell culture supernatant

Principle:

The method employs sandwich ELISA technique. Human Coronavirus antigen is pre-coated onto microwells. Samples and standards are pipetted into microwells and IgG present in the sample are bound by the capture antigen. HRP labelled anti-Human IgG is added 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 Coronavirus 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 Coronavirus Antigen Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Human Coronavirus Antigen	1 x 96 wells
Human Coronavirus IgG Standard	Lyophilized Human Coronavirus IgG Standard Concentration – 1000 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. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. 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. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or lower temperature. Avoid repeated freeze-thaw cycles. If the use of original supernates 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.

Should you desire to inactivate the virus, use a (5X) Lysis Buffer (optional, not provided). Add 1/5 volume of (5X) Lysis Buffer to sample (i.e. add 50 ul (5X) Lysis Buffer to 200 ul sample). Vortex well.

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.

Respiratory Sample - Centrifuge samples for 20 minutes at 10000 x g at 2-8°C. Collect supernatant and carry out the assay immediately.

Preparation before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Serum and Plasma Samples should be diluted 1:1000 (v/v) for optimal recovery, (for example 1 ul sample + 999 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 Standards (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
1000 ng/ml	Original Standard	Original Standard provided in the Kit + 1ml of Standard Diluent
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

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 IgG.
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 Room Temperature (18-25ºC).

3. Aspirate and wash plate **5 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 **Anti-Human IgG:HRP Conjugate** to each well.

5. Seal plate and incubate for 60 minutes at Room Temperature (18-25°C).
6. Wash plate **5 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 Human Coronavirus IgG 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.

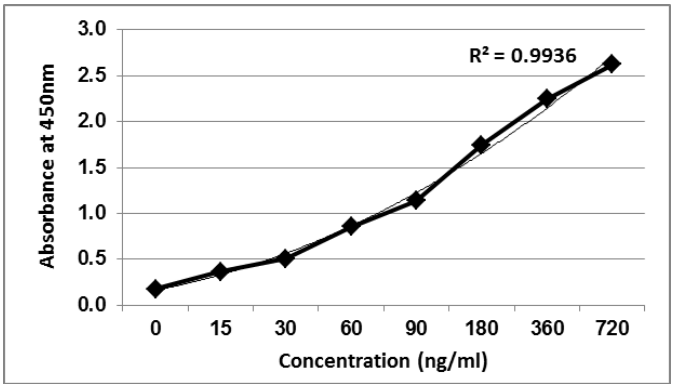
Software which is able to generate a polynomial regression (2nd order) or a cubic spline curve-fit is best recommended for automated results.

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.
- If the absorbance value is equivalent or higher than the 720 ng/ml standard.

Typical Data

Standards (ng/ml)	Abs	Abs	Mean Abs	Interpolated Concentration	% Recovery
0	0.184	0.170	0.177	--	--
15	0.359	0.366	0.363	17.1	113.9
30	0.503	0.511	0.507	29.0	96.7
60	0.843	0.866	0.854	59.4	99.1
90	1.100	1.180	1.140	89.2	99.2
180	1.761	1.734	1.747	184.2	102.4
360	2.256	2.234	2.245	351.8	97.7
720	2.605	2.639	2.622	728.9	101.2

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.

Standards Range:
0 ng/ml - 720 ng/ml

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 12 ng/ml

Specificity:
The antibodies used in the kit are monoclonal antibodies specific for human SARS-CoV. The standard used is a human SARS-CoV IgG. Cross Reactivity may be observed to SARS-Cov-2.

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 with low (15 ng/ml), medium (60 ng/ml) and high (720 ng/ml) concentrations. 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%	<12%
Medium	<11%	<10%
High	<12%	<10%

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.

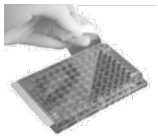
Components (2-8ºC) → 30 mins RT Before Use 

→ 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

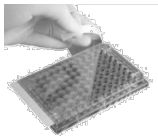
3.  Pipette **100 ul Standards and diluted Samples** into the respective wells.

4. Cover plate  and **incubate** for 

at Room Temperature.

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

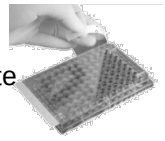
6.  Pipette **100 ul Anti-Human IgG:HRP Conjugate** into each well.

7. Cover plate  and **incubate** for 

at Room Temperature.

8.  Aspirate and wash wells 5 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 ng/ml 0 ng/ml			
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

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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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