GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgG Antibody spike protein Qualitative ELISA

REF : KBVH015-8

Ver 3.1

IVD

Enzyme Immunoassay for the Qualitative Determination of IgG Antibodies to Human SARS-CoV-2 (Covid-19) in human serum and plasma.

IVD	**For In-Vitro Diagnostic**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

For *Invitro Diagnostic use only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN Pudgala LLP is strictly prohibited.*

REF **KBVH015-8**  **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 ELISA kits which employ only one antibody.

Clinical Significance:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV) belongs to the family of coronaviruses and, like SARS-CoV, is classified in the genus Betacoronavirus. SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected persons. Health care personnel and family members are especially at risk of infection. The zoonotic reservoir of the virus appears to be bats.

The incubation time of SARS-CoV is three to seven, maximally 14 days. The most common symptoms of SARS-CoV-2 infection are fever, coughing, breathing difficulties and fatigue. In most patients the infection manifests with symptoms of a mild febrile illness with irregular lung infiltrates. Some patients, especially elderly or chronically ill patients, develop acute respiratory distress syndrome (ARDS). Reported case fatality rates depend on geographic location, age, and comorbidities.

Suitable methods for the diagnosis of SARS-CoV-2 infections are detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) or of viral protein by ELISA primarily in sample material from the upper (nasopharyngeal or oropharyngeal smear) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, nasopharyngeal secretion, oropharyngeal secretion, etc). The determination of antibodies enables confirmation of recent or prior SARS-CoV-2 infection in patients with typical symptoms and in suspected cases. It also contributes to monitoring and outbreak control. Cross reactions with antibodies within the genus Betacoronavirus have been described.

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 ELISA kits which employ only one antibody.

Intended Use:

The GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgG ELISA kit is used as an analytical tool for qualitative estimation of IgG antibodies to Human SARS-CoV-2 (Covid-19) spike proteins in human serum and plasma (K+-EDTA, Li+-heparin, Na+-citrate).

The GENLISA™ Human Anti-SARS-CoV-2 ELISA (IgG) is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity.

The GENLISA™ Human Anti-SARS-CoV-2 ELISA (IgG) should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under ICMR. Results are for the detection of SARS CoV-2 antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within India are required to report all positive results to the appropriate public health authorities.

The sensitivity of GENLISA™ Human Anti-SARS-CoV-2 ELISA (IgG) early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary. False positive results for the GENLISA™ Human Anti-SARS-CoV-2 ELISA (IgG) may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

Principle:

The method employs sandwich ELISA technique. Human SARS-CoV-2 spike protein is pre-coated onto microwells. Samples and standards are pipetted into microwells and IgG Antibodies to human SARS-CoV-2 (Covid-19) present in the sample are bound by the protein antigen. After incubation the wells are washed and followed by HRP-conjugated Detection IgG 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 IgG -Human Anti-SARS-CoV-2 (Covid-19) in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Recombinant SARS-CoV-2 (Covid-19) spike protein Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with recombinant SARS-CoV-2 (Covid-19) spike protein	1 x 96 wells
Positive Control (Human Anti-SARS-CoV-2 spike protein) (concentrated)	Positive Control (Human Anti-SARS-CoV-2 spike protein), (concentrated. lyophilized)	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) Assay Diluent	Buffered protein base with preservative thiomersol < 0.01%	2 x 50 ml
Control Diluent	Buffered protein base with preservative thiomersol < 0.01% 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	0.73M Phosphoric 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. 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 Assay diluent (1X)) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted with Assay 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

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.

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. **Postive Control** Preparation: Reconstitute the concentrated lyophilized vial with 1 ml of Control Diluent to obtain **Positive Control**. Keep the vial for 15 mins with gentle agitation and then run the assay procedure.
5. **Negative Control** Preparation: Use the residual 1 ml of Control Diluent provided with the kit as Negative Control.

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. High Dose Hook Effect may be observed in samples with very high concentrations of Human Anti-SARS-CoV-2 (Covid-19) IgG. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Anti-SARS-CoV-2 (Covid-19) IgG present in the sample.
3. Avoid assay of Samples containing sodium azide (NaN3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Anti-SARS-CoV-2 (Covid-19).
4. It is recommended that the Controls and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. 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.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. Pipette **100 ul** of **Positive, Negative Controls** or **diluted Samples** in duplicates to the respective wells

2. Seal the plate and incubate for 1 hour at Room Temperature (18-25ºC).

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 the plate and incubate for 1 hour at Room Temperature (18-25°C).
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 15 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.

Interpretation of Results:

Read the positive control, negative control and sample wells on microtitre plate reader at 450nm. The results should be interpreted in regard to anamnesis, further clinical observations and results of other diagnostic investigations. Negative results do not preclude acute SARS-Cov-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.

Positive Samples	**OD > 1.0**
Negative Samples	**OD < 0.5**
Unequivocal/Grey Zone Samples *	**OD < 1.0 and > 0.5**

Note:
1. Incase your samples show false positive or false negative results; we recommend to increase the interpretation of the Positive Samples to OD > 1.5. (Classen et al.1987).
2. Incase your samples report absorbance unequivocally, we recommend to report such samples as unequivocal samples in absence of clinical interpretation

Validity of the Test:

The use of controls allows validation of the test. The test is valid if the following conditions are met,

Positive Control Value	**OD > 1.0**
Negative Control Value	**OD < 0.5**

In case the control value is out of range, we recommend you to repeat the assay.

Since IgG antibodies to SARS-CoV-2 generally become detectable beginning 10-14 days following infection or vaccination, the borderline samples may indicate the beginning of seroconversion, i.e. the patient is possibly developing antibodies. Therefore, it is strongly recommended to repeat sample drawing and testing around 14 days after the first sample drawing

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:

Sensitivity:
Limit Of Detection: There is no standard reference SARS-CoV-2 antigen material available; accordingly, absolute analytical sensitivity cannot be calculated. Based on the kit working standards the LOD is 10 AU/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).

Cross-reactivity of non-SARS-CoV-2 specific antibodies against SARS-CoV-2 spike proteins in Anti-SARS-CoV-2 ELISA KBVH015-8 was examined using sera with known antibodies against confirmed past infections.

N	Antibody Positive Sera	Anti-SARS-CoV-2 ELISA #KBVH015-8
1	Beta Corona HKU1*	Negative
4	VCV	Negative
5	HCV	Negative
4	HAV	Negative
3	HBV	Negative
4	EBV	Negative
5	CMV	Negative
5	HSV	Negative

*The patient was tested PCR positive for Beta Corona HKU1 and PCR negative for SARS-CoV-2. Four weeks after PCR testing a serum sample was drawn from the patient and found to be negative in the Anti-SARS-CoV-2 ELISA.

Clinical Sensitivity and Specificity:
Three (3) lots of KBVH015-8 were validated at National Insititute of Virology, (an Indian Council of Medical Research unit), India with known clinical samples. Clinical sensitivity was estimated at ~95% and clinical specificity at ~96.33%.

Lot No	01967 (IgG)			01968 (IgG)			01969 (IgG)		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	90	4	94	92	3	95	92	4	96
Negative	10	96	106	8	97	105	8	96	104
Total	100	100	200	100	100	200	100	100	200

Lot No	01967 (IgG)		01967 (IgG)		01967 (IgG)	
	Estimate %	95%CI	Estimate %	95%CI	Estimate %	95%CI
Sensitivity	90	82.38 - 95.10	92	84.84 - 96.48	92	94.94 - 96.48
Specificity	96	90.07 - 98.90	97	91.48 - 99.38	96	90.07 - 98.90

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 AU/ml), medium (180 AU/ml) and high (720 AU/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	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

Recovery:
Human sera and plasma were measured with two replicates and two runs (n = 5). The human 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)	87 - 112
Plasma EDTA (n=5)	85 - 114
Plasma Heparin (n=5)	86 - 114

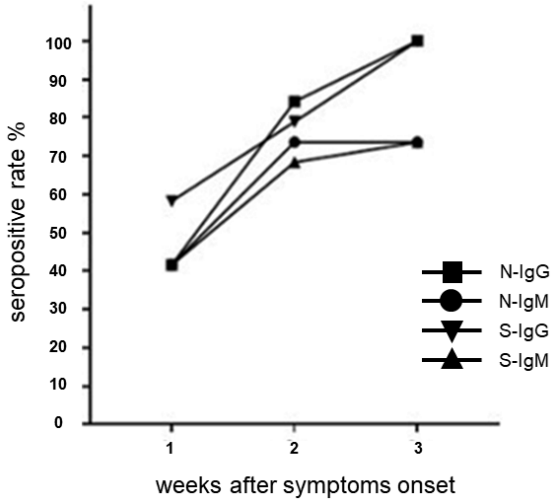
Note: Serum and Plasma were diluted using 1X Assay Diluent provided with the kit

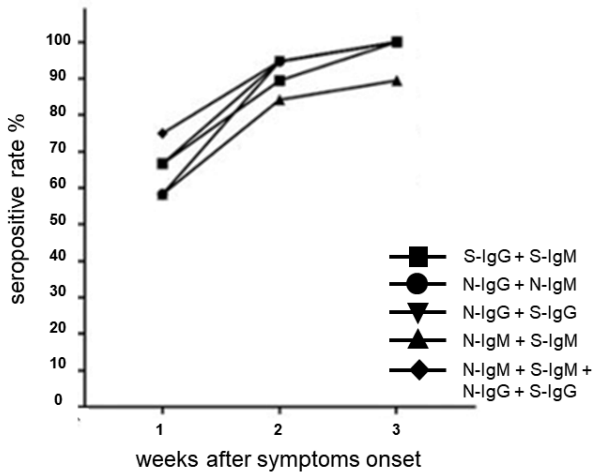
Assay Comparison:

To demonstrate the identity of the Anti-SARS-CoV-2 ELISA and a commercially available COVID-19 IgG / IgM Rapid Test, 9 serum samples were measured using both assays. In comparison, the results of the IgG tests show an increased sensitivity of the anti-SARS-CoV-2 ELISA.

Sample No.	Anti-SARS-CoV-2 ELISA	COVID-19 IgG / IgM Rapid Test
1	positive +	negative
2	positive +	positive
3	positive +	negative
4	negative -	negative
5	borderline +	slightly positive
6	positive +++	positive
7	negative -	negative
8	negative -	negative
9	positive ++	positive

Longitudinal Reponse of Antibodies to SARS-CoV-2.
Antibodies response to the Nucleocapsid Proteins and the Spike Proteins of IgG and IgM:





Weeks	N-IgM	N-IgG	S-IgM	S-IgG	N-IgM + N-IgG	S-IgM + S-IgG	N-IgM + S-IgM	N-IgG + S-IgG	N-IgM+ S-IgM+ N-IgG+ S-IgG
1	41.7	41.7	41.7	58.3	58.3	66.7	58.3	66.7	75
2	73.7	84.2	68.4	78.9	94.7	89.5	84.2	94.7	94.7
3	73.7	100	73.7	100	100	100	89.5	100	100

Safety Precautions:

- **This kit is For In-Vitro Diagnostic 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.

References:

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M Letko, A Marzi, V Munster
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EMERGING MICROBES AND INFECTIONS JOURNAL
Accepted author version posted online: 01 May 2020

Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients
Baoqing Sun, Ying Feng, Xiaoneng Mo, Peiyan Zheng, Qian Wang, Pingchao Li, Ping Peng, Xiaoqing Liu, Zhilong Chen, Huimin Huang, Fan Zhang, Wenting Luo, Xuefeng Niu, Peiyu Hu, Longyu Wang, Hui Peng, Zhifeng Huang, Liqiang Feng, Feng Li, Fuchun Zhang, Fang Li, Nanshan Zhong & Ling Chen

https://doi.org/10.1080/22221751.2020.1762515

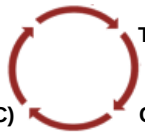
SCHEMATIC ASSAY PROCEDURE

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

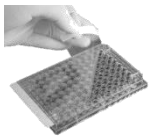
Components (2-8ºC) → 30 mins RT Before Use → 

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

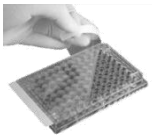
3.  Pipette **100 ul Positive and Negative Controls and diluted Samples** into the respective wells.

4. Cover plate  and incubate for 

at Room Temperature.

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

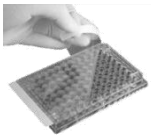
6.  Pipette **100 ul Goat Anti-Human IgG:HRP Conjugate** into each well.

7. Cover plate  and incubate for 

at Room Temperature.

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	Negative Control Negative Control			
1B 2B	Positive Control Positive Control			
1C 2C	Sample Sample			
1D 2D	Sample Sample			
1E 2E	Sample Sample			
1F 2F	Sample Sample			
1G 2G	Sample Sample			
1H 2H	Sample Sample			

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Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP 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 Pudgala LLP shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Pudgala LLP 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 Pudgala LLP be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. 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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