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

REF : KBVH015-8

Ver 1.2

RUO

Enzyme Immunoassay for the Quantitative estimation of IgG Antibodies to Human SARS-CoV-2 (Covid-19) in 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 **KBVH015-8** **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.

There is no standard reference SARS-CoV-2 antigen material available; accordingly, absolute analytical sensitivity cannot be calculated. We have used a polyclonal antibody as standard and quantified for the total protein content of the antibodies raised as 1000 AU/ml. The SARS-CoV-2 Antibody has not been defined or standardized worldwide. Hence this assay uses standards optimized for the linear range in the assay as AU/ml. The kit shows a typical titration curve when used in dilutions of stable below.

Standard Concentration	Standard Titre Dilution
15 AU /ml	1:66,666 titre
30 AU /ml	1:33,333 titre
60 AU /ml	1:16,667 titre
90 AU /ml	1:11,111 titre
180 AU /ml	1:5556 titre
360 AU /ml	1:2778 titre
720 AU /ml	1:1389 titre

The assay allows the quantitative determination of samples of an unknown concentration titer (immunological titer) and the calibration of the kit standards.

It is also important to note that the polyclonal antibody used as standard is interpreted as ng/ml. However, in the absence of standardization of the SARS-CoV-2 antibodies worldwide, the same are expressed as AU/ml in the kit.

Intended Use:

The GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgG ELISA kit is used as an analytical tool for quantitative estimation of IgG antibodies to Human SARS-CoV-2 (Covid-19) in human serum and plasma, respiratory specimens and cell culture supernatant.

Principle:

The method employs sandwich ELISA technique. Human SARS-CoV-2 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 Anti-Human 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:

1. Recombinant SARS-CoV-2 (Covid-19) spike protein Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Anti-Human SARS-CoV-2 (Covid-19) Standards, (0.5 ml/vial) - 0, 15, 30, 60, 90, 180, 360 and 720 AU/ml
3. Anti Human IgG:HRP Conjugate – 12 ml
4. (5X) Assay Diluent - 50 ml
5. (20X) Wash Buffer - 25 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12 ml

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 1:5000 (v/v) for optimal recovery, (for example 1 ul sample + 4999 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*

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

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 **Assay Diluent (1X)**; dilute **50 ml** of **5X** Assay Diluent in **200 ml of DI water**.
4. To make **Wash Buffer (1X)**; dilute **25 ml** of **20X** Wash Buffer in **475 ml of DI water**.

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 Anti-Human SARS-CoV-2 (Covid-19) IgG. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-Human SARS-CoV-2 (Covid-19) IgG present in the sample.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-Human SARS-CoV-2 (Covid-19).
4. It is recommended that the Standards 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 Standards and Samples.

Assay Procedure:

1. Pipette **100 ul** of **Standards** and **diluted Samples** in duplicates to the respective wells.

2. Seal 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 **Anti-Human IgG:HRP Conjugate** to each well.

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

6. Wash plate 4 times with **Wash Buffer (1X)** as in step 2.

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

8. Incubate the plate at Room Temprature (18-25°C) for 15 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

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 Anti-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.

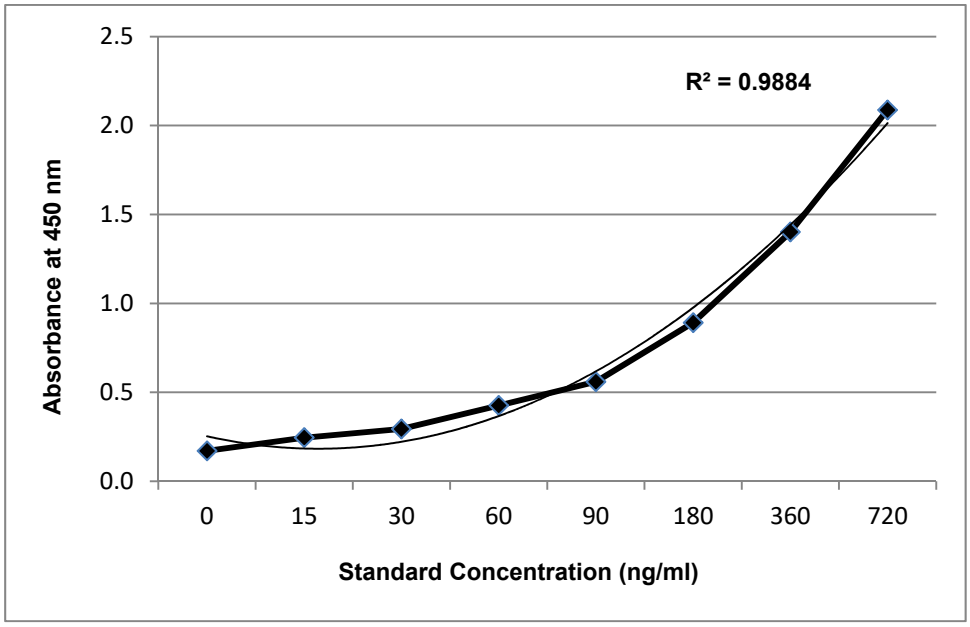
Software which is able to generate a polynomial regression (2^{nd} order) or a cubic spline curve-fit is best recommended for automated results.

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

Typical Data

Standard Concentration (AU/ml)	Abs 1	Abs 2	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.184	0.157	0.171	-	-
15	0.254	0.236	0.245	16.9	112.6
30	0.304	0.283	0.294	28.0	93.3
60	0.441	0.410	0.426	58.6	97.7
90	0.558	0.561	0.559	91.1	101.2
180	0.886	0.897	0.891	181.7	100.9
360	1.407	1.396	1.401	358.2	99.5
720	2.079	2.097	2.088	720.7	100.1

Typical Data



note: ng/ml = AU/ml

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.

Traceability:
There are no reference standards for SARS-Cov-2 Antibody. The results are reported in AU/ml 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 IgG Antibodies to SARS-CoV-2 present in matrix. The Standards / Calibrator Range is 0 - 720 AU/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 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

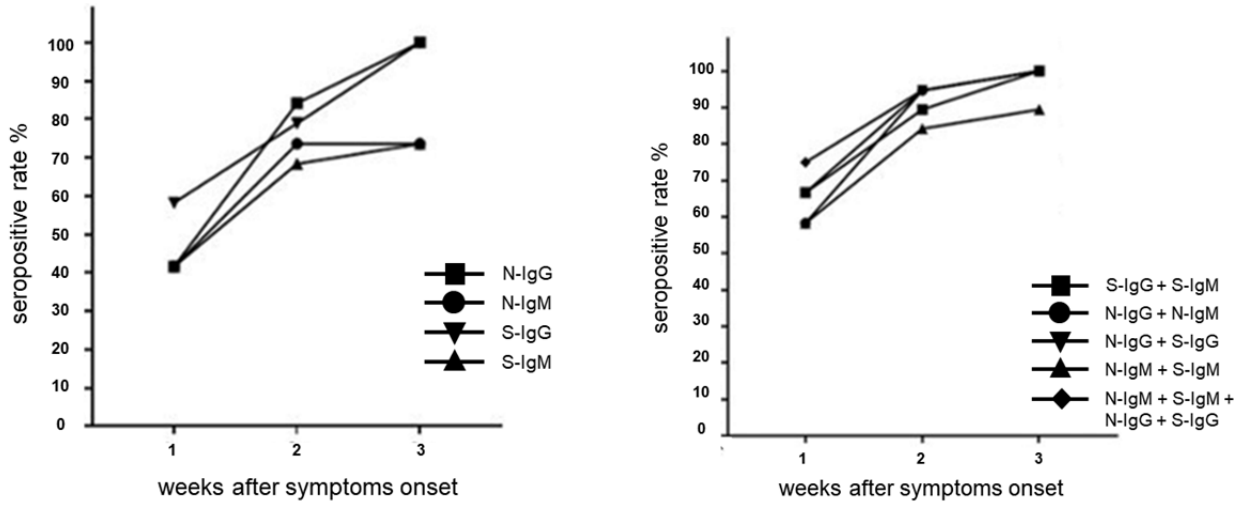
Note: Serum and Plasma were diluted using Standard Diluent provided with the kit

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:

figure a and b



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

Reference:

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

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.

- 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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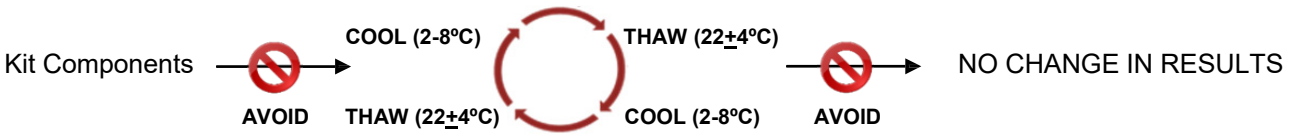
Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses
M Letko, A Marzi, V Munster
- Nature microbiology, 2020 - nature.com

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 Controls and diluted Samples** into the respective wells.

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

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

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

7. Cover plate and **incubate** for 60min 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 15min at Room Temperature.

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

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

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