






GENLISA™ Human Anti-SARS-CoV-2 (Covid-19) IgM Antibody to nucleoprotein ELISA

REF : KBVH015-20

Ver 1.1

RUO

Enzyme Immunoassay for the Quantitative Estimation of Anti-SARS-CoV-2 IgM antibodies to nucleoprotein 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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 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. Double antibodies are used in this kit.

Intended Use:

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

Principle:

The method employs sandwich ELISA technique. Human SARS-CoV-2 nucleoprotein is pre-coated onto microwells. Samples and standards are pipetted into microwells and IgM 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 Antigen 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 IgM 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:

Part	Description	Qty
Recombinant SARS-CoV-2 Nucleoprotein Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with SARS-CoV-2 Nucleoprotein.	1 x 96 wells
Anti-SARS-CoV-2 Nucleoprotein IgM Antibody Standard	Anti-SARS-CoV-2 Nucleoprotein IgM Antibody Standard (lyophilized, concentrated - 1000 ng/ml)	2 vials
Goat Anti-Human IgM:HRP Conjugate	Goat Anti-Human IgM:HRP Conjugate prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	2 x 50 ml
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 recommended storage temperature indicated on the component label.
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 1:1000 (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 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 Anti-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 compromise 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 **Samples** to the respective wells.
2. Seal the plate and incubate for 1 hour at Room Temperature (18-25°C).
3. 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 IgM: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)** 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.
7. Pipette **100 ul** of **TMB Substrate solution** in all wells.
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.

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-SARS-CoV-2 Nucleoprotein IgM 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.

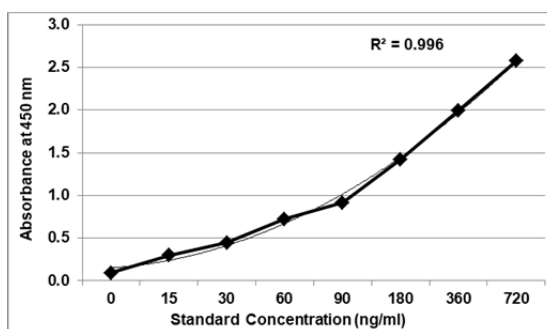
Note:

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.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.096	0.093	0.095	—	—
15	0.305	0.297	0.301	15.7	104.9
30	0.453	0.437	0.445	29.5	98.2
60	0.727	0.724	0.725	61.4	102.4
90	0.951	0.875	0.913	87.3	97.0
180	1.435	1.409	1.422	181.7	100.9
360	2.048	1.947	1.998	360.2	100.1
720	2.591	2.562	2.576	719.1	99.9

Typical Graph



Abs = absorbance at 450nm

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 Nucleoprotein material available; accordingly, absolute analytical sensitivity cannot be calculated. Based on the kit working standards the LOD is 10 ng/ml

Specificity:

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). The kit antibodies are specific to Nucleoprotein of the SARS-CoV-2 virus.

Traceability:

There are no reference standards for SARS-Cov-2 Antibody. The results are reported in ng/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 Human IgM Antibodies to SARSCoV-2 present in respiratory specimens and serum. The Standards / Calibrator Range is 0 - 720 ng/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. 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%	<15%
Medium	<12%	<12%
High	<12%	<12%

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)	85 - 115
Plasma EDTA (n=5)	80 - 120

Note: Serum/Plasma was diluted using Sample Diluent provided with the kit

Reference:

EMERGING MICROBES AND INFECTIONS JOURNAL

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.
- 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.



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 not manufactured 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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to nucleoprotein ELISA**

KRISHGEN BioSystems

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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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.

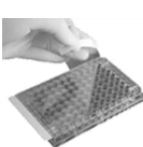




3.  Pipette **100 ul Standards** and **diluted Samples** into the respective wells.

4.  Cover plate and **incubate** for  at Room temperature

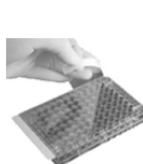

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X)**.

6.  Pipette **100 ul Goat Anti-Human IgM: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.

SYMBOLS KEY

	SARS-CoV-2 Nucleoprotein Coated Microtiter Plate
	Anti-SARS-CoV-2 Nucleoprotein IgM Antibody Standard
	Goat Anti-Human IgM:HRP Conjugate
	Standard Diluent
	Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature