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

REF: KBVH015-19

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Screening of IgM Antibodies to Human SARS-CoV-2 (Covid-19) in respiratory specimens and human serum.

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 **KBVH015-19** **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. 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 respiratory specimens and human serum.

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 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:

1. Recombinant SARS-CoV-2 (Covid-19) Spike protein Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Anti-SARS-CoV-2 (Covid-19) Standards - 0, 15, 30, 60, 90, 180, 360 and 720 AU/ml
3. Anti-Human IgM:HRP Conjugate - 1 vial
4. (20X) Assay Diluent - 8 ml
5. (20X) Wash Buffer - 25 ml
6. TMB Substrate - 13 ml
7. Stop Solution - 8 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 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.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Respiratory Specimens-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. It is recommended to follow the CDC (Centre for Disease Control), Atlanta, USA guidelines for specimen handling and treatment. (https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf)

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 **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) IgM. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-Human SARS-CoV-2 (Covid-19) IgM present in the sample.
3. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
4. Anti-Human SARS-CoV-2 (Covid-19) IgM concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
5. 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).
6. It is recommended that all Controls and Samples be assayed in duplicates.
7. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
8. 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.
9. The plates should be read within 30 minutes after adding the Stop Solution.
10. 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. Seal plate and incubate for 2 hours at Room Temperature (18-25ºC).

2. Aspirate and wash plate 3 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.

3. Add 100 ul of **Anti-Human IgM:HRP Conjugate** to each well.

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

5. Wash plate 3 times with **Wash Buffer (1X)** as in step 2.

6. Pipette 200 ul of **TMB Substrate solution** (premixed Substrate A and Substrate B).
7. Incubate in the dark for 20 minutes at Room Temperature. Positive wells should turn bluish in color.
8. Stop reaction by adding 50 ul of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
9. Read absorbance at 450 nm within 20 minutes of stopping reaction.

Interpretation of the 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 (Covid-19) IgM Antibody to spike protein 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.

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.

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

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 Human IgM Antibodies to SARSCoV-2 present in respiratory specimens and serum. The Standards / Calibrator Range is 0 - 720 AU/ml (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 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%	<12%
Medium	<5%	<8%
High	<5%	<8%

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
Plasma Heparin (n=5)	80 - 120

Reference:

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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

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