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GENLISA™ Human SARS-CoV-2 (Covid-19) nucleoprotein Qualitative ELISA

REF : KBVH015-5

Ver 1.0

RUO

Enzyme Immunoassay for the Qualitative nucleoprotein Antigen Determination of 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 KBVH015-5 **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 SARS-CoV-2 (Covid-19) Nucleoprotein Antigen ELISA kit is used as an analytical tool for qualitative antigen determination of Human SARS-CoV-2 (Covid-19) in respiratory speciments and human serum.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human SARS-CoV-2 (Covid-19) present in the sample are bound by the capture antibodies. After incubation the wells are washed and followed by HRP-conjugated Detection 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 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. SARS-CoV-2 (Covid-19) Nucleoprotein Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Positive Control, recombinant Human SARS-CoV-2 (Covid-19) Nucleoprotein - 1 vial
3. Negative Control - 1 vial
4. SARS-CoV-2 (Covid-19) Antibody:HRP Conjugate (0.2 mg/ml) - 1 vial
5. (20X) Assay Diluent - 8 ml
6. (20X) Wash Buffer - 25 ml
7. Substrate A - 13 ml
8. Substrate B - 13 ml
9. 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 **Wash Buffer (1X)**; dilute **20 ml of 20X Wash Buffer in 380 ml of DI water**.
4. To make **Assay Diluent 1X**: Dilute **5 ml of 20 X Assay Diluent in 95 ml of DI water.**
5. **SARS-CoV-2 (Covid-19) Antibody:HRP Conjugate:** Centrifuge at 10,000 x g for 20 seconds. Dilute to work concentration of **0.5 ug/ml** in Dilution Buffer before use.
6. **Substrate Solution:** Substrate A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light.
7. **Positive Control**: Reconstitute the lyophilized vial with 1 ml of Distilled 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 Human SARS-CoV-2 (Covid-19). High Dose Hook Effect is due to excess of antibody for very high concentrations of Human SARS-CoV-2 (Covid-19) 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. Human SARS-CoV-2 (Covid-19) 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 Human SARS-CoV-2 (Covid-19).
6. It is recommended that all Standards 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 **Controls** 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 **diluted SARS-CoV-2 (Covid-19) Antibody: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 Results:

Cut Off Value = Mean for Negative/Blank + 0.1

Positive Sample Value = OD > Cut Off value
Negative Sample Value = OD < Cut Off value

Calculation for Cut Off Values:

Read the sample and blank/negative control wells on microtitre plate reader at 450nm. The OD (Optical Density) of NC (Negative Control) in triplicate should be used for calculating the mean and standard deviation. This is the $Negative_{mean}$. The cut-off for positives is equal to a value greater than ($Negative_{mean}$ + Standard Deviation).

Formula:
Positive Sample Value = OD > ($Negative_{mean}$ + SD)

For example –

Sample Type	Absorbance #1	Absorbance #2	Absorbance #3	Mean
Negative	0.131	0.128	0.130	0.129
Standard Deviation	0.131-0.129	0.128-0.129	0.130-0.129	
	= 0.002	= -0.001	=0.001	

Mean Standard Deviation = $\sqrt{(0.002)^2 + (-0.001)^2 + (0.001)^2 / 3} = 0.0014$

Therefore Cut-off = Mean + 3*SD
= 0.129 + 3* 0.0014
= 0.129 + 0.0042
= 0.133

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using 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 SARS-CoV-2 (Covid-19) Nucleoprotein 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 Human SARS-CoV-2 (Covid-19) Concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate 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.

Laboratory Values:
Positive Sample: Absorbance value greater than Cut Off Value.
Negative Sample: Absorbance value lesser than Cut Off Value
Intermediate Sample: Absorbance value near Cut Off Value. In this case, the sample should be re-tesed and confirmation done by Real time PCR method using our kit (#KBRT010)

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:
The antibodies used in the kit for capture and detection are highly specific rabbit monoclonal against SARS-Coronavirus (Covid-19) Nucleoprotein. The calibrators used are recombinant protein of SARS-Coronavirus (Covid-19) Nucleoprotein.

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 less than 42.5 pg/ml.

Linearity:
Known quantity of the protein was spiked in human serum and cell culture supernatant and concentration analyzed. The mean recoveries showed the samples fall within the dilutional linearity of upto 1:16.

Dilution	Particulars	Serum Matrix	PBS Buffer Matrix
1:2	recovery of detected analyte	81 %	101 %
1:4	recovery of detected analyte	94 %	102 %
1:8	recovery of detected analyte	107 %	97 %
1:16	recovery of detected analyte	126 %	93 %

Precision:
Intra-Assay
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.
Inter-Assay: Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

	Intra-Assay			Inter-Assay		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/ml)	531	1105	2300	590	1246	2443
SD	65.01	99.66	104.52	59.00	159.33	176.47
CV (%)	12.2 %	9.0 %	4.5 %	10.0 %	12.8 %	7.2 %

Recovery:
SARS-CoV-2 (Covid-19) Nucleoprotein was spiked at different levels to measure mean recovery.

Sample	Mean % Recovery	Range
Serum (n=3)	81	78-84 %
PBS Buffer Matrix (n=3)	117	110-127 %

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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