Human SARS-CoV-2 (Covid-19) Nucleoprotein ELISA Kit

Cat#: orb638753 [Product Manual]

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) IgG ELISA kit is used as an analytical tool for screening for IgG antibodies to Human SARS-CoV-2 (Covid-19) in respiratory speciments 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 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 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 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) nucelocapsid protein Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Positive Control (Anti-Human SARS-CoV-2 (Covid-19)) - 1 vial
3. Negative Control - 1 vial
4. Recombinant SARS-CoV-2 (Covid-19):HRP Conjugate - 1 vial
5. (20X) Assay Diluent - 8 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 13 ml
8. 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 main kit components at 2-8°C.
2. Store recombinant Standard at -20°C. Aliquot recombinant protein and detection antibody into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
4. 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.

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.

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. 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) IgG 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 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 SARS-CoV-2 (Covid-19):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:

Cut Off Value = Mean for Negative + 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 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 Negativemean. The cut-off for positives is equal to a value greater than (Negative mean + Standard Deviation).

Formula:

Positive Sample Value = OD > (Negativemean + 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 = $\sqrt{(0.002)^2 + (-0.001)^2 + (0.001)^2 \quad / 3} = 0.0014$

Therefore Cut-off = Mean + 3*SD

= 0.129 + 3* 0.0014

= 0.129 + 0.0042

= 0.133

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 antigens used in the (Covid-19). The positive SARS-CoV-2 (Covid-19).

kit for capture and detection are recombinant nucelocapsid proteins for SARS-CoV-2 control used is a specific monoclonal antibody towards the nucleocapsid protein of

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

Biorbyt Ltd 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 Biorbyt Ltd, or against damages resulting from such non-Biorbyt Ltd made products or components. Biorbyt Ltd passes on to customer the warranty it received (if any) from the maker thereof of such non Biorbyt made products or components.

This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Biorbyt Ltd.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Biorbyt Ltd shall be to repair or replace the defective Products in the manner and for the period provided above. Biorbyt Ltd 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 Biorbyt Ltd be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Biorbyt Ltd 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.

Biorbyt Ltd.
5 Orwell Furlong, Cowley Road,Cambridge, Cambridgeshire
CB4 0WY, United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859 353 | Fax: +44(0)1223 280 240

Biorbyt LLC
Suite 103, 369 Pine Street, San Francisco
California 94104, United States
Email: info@biorbyt.com | Phone: +1 (415)-906-5211 | Fax: +1 (415) 651 8558