KRISHGEN BioSystems

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GENLISA™ SARS-CoV-2 (Covid-19) (Wild Type+South African Variant) RBD Antigen Quantitative TITRATION ELISA

REF : KBVH015-41

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Estimation of SARS-CoV-2 (Wild Type+South African Variant) RBD antigen in cell culture supernatant and biological preparations including vaccines.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

REF **KBVH015-34** 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.

Several new variants of SARS-CoV-2 virus have emerged in recent months. The U.K. variant B.1.1.7, the Brazil variant P.1, the South Africa variant B.1.351, and the Indian variant B.1.617 are particular concerning because of their high prevalence. A subset of the mutations identified in the RBD domain of the spike protein occurs in more than one strain, These convergent mutations are of high interest because they may be the cause of the increased transmissibility.

The GENLISA™ SARS-CoV-2 (Covid-19) (Wild Type+South African Variant) RBD Antigen Quantitative TITRATION ELISA kit employs antibodies specific to WT, beta (B.1.351, B.1.351.2, B.1.351.3). The antibodies used in the kit also show cross reactivity to the gamma variant, B.1.617, and B.1.429 variants of SARS-CoV-2. The standards used in the kit are a specific mix of spike RBD (K417N, E484K, N501Y (B.1.351 | Beta Variant)) and spike RBD (WT).

Intended Use:

The GENLISA™ SARS-CoV-2 (Covid-19) (Wild Type+South African Variant) RBD Antigen Quantitative TITRATION ELISA kit is used as an analytical tool for quantitative estimation of SARS-CoV-2 RBD antigen in cell culture supernatant and biological preparations like vaccines.

Principle:

The method employs sandwich ELISA technique. SARS-CoV-2 Spike RBD Antibody is pre-coated onto microwells. Samples and standards are pipetted into microwells and SARS-CoV-2 RBD Antigen (Wild Type & South African) present in the sample are bound by the antibodies. After incubation the wells are washed and followed by addition of HRP-conjugated Detection Antibody into each well 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 SARS-CoV-2 RBD Antigen (2019-nCoV) 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 Spike Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. SARS-CoV-2 Spike RBD Standard (conc., lyophilized 1000 ng/ml) - 2 vials.
3. Anti-SARS-CoV-2:HRP Conjugate - 12 ml
4. Standard Diluent - 10 ml
5. Sample Diluent - 2 x 40 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
8. Instruction Manual

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:

Cell Culture Supernatant- Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.

Biological Preparations including Vaccines- if prepared solutions, dilute to expected concentration within the kit assay range using Sample Diluent provided in the kit. For lyophilized preparations including vaccines, reconstitute using the Sample Diluent. Keep for 5 mins and mix well. Use the Sample Diluent for further dilution to bring the sample within the expected assay range of the kit.

Note: the kit has not been validated for excipients and other reagents including adjuvants. If a poor recovery is seen, dilute the samples further to negate the matrix effect.

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 SARS-CoV-2 RBD Antigen (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 compromisation 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 (18-25ºC).

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 **Anti-SARS-CoV-2: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 SARS-CoV-2 Spike RBD Antigen (Wild Type & South African Variant) 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), 4PL 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.

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.
- 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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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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