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# GENLISA™ Mouse Anti-SARS-CoV-2 (Covid-19) IgG2b Antibody to whole spike protein Quantitative TITRATION ELISA

**REF** : KBVH015-55

Ver 1.0

**RUO**

Enzyme Immunoassay for the Quantitative Estimation of IgG2b Antibodies to SARS-CoV-2 (Covid-19) whole spike protein in Mouse serum

<b>RUO</b>	For Research Use Only	<b>REF</b>	Catalog Number
	Store At	<b>LOT</b>	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.

**Intended Use:**

The GENLISA™ Mouse Anti-SARS-CoV-2 (Covid-19) IgG2b Antibody to whole spike protein Quantitative TITRATION ELISA kit is used as an analytical tool for quantitative estimation of Anti-SARS-CoV-2 (2019-nCoV) whole spike protein IgG2b antibodies in Mouse serum.

**Principle:**

The method employs indirect sandwich ELISA technique. SARS-CoV-2 whole spike protein is pre-coated onto microwells. Samples and standards are pipetted into microwells and Antibodies to SARS-CoV-2 (2019-nCoV) whole spike protein present in the sample are bound by the protein antigen. After incubation the wells are washed and followed by addition of HRP-conjugated Detection IgG2b 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 Mouse Anti-SARS-CoV-2 (2019-nCoV) whole spike protein IgG2b 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 (Covid-19) Spike protein Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Recombinant SARS-CoV-2 (Covid-19) whole spike protein	1 x 96 wells
Anti-SARS-CoV-2 Spike protein Antibody Standard	Lyophilized Anti-SARS-CoV-2 whole spike protein Antibody Standard (concentrated – 1000 ng/ml)	2 vials
Goat Anti-Mouse IgG2b:HRP Conjugate	Goat Anti-Mouse IgG2b: HRP Conjugate prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
1X Sample Diluent	Buffered protein base with preservative thiomersol < 0.01%	2 x 50 ml
1X Standard Diluent	Buffered protein base with preservative thiomersol < 0.01% with 1:1000 dilution Mouse 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 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.



**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 720 ul of original **Standard (1000 ng/ml)** with 280 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 + 1ml Standard Diluent
720 ng/ml	Standard No.7	720 ul Original Standard (1000 ng/ml) + 280 ul Standard Diluent
360 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
180 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
90 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
60 ng/ml	Standard No.3	666.7 ul Standard No.4 + 333.3 ul Standard Diluent
30 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
15 ng/ml	Standard No.1	500 ul Standard No.2 + 500 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 ( $\text{NaN}_3$ ), 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 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- Mouse IgG2b: HRP Conjugate** to each well.
5. Seal 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 Mouse Anti-SARS-CoV-2 whole spike protein IgG2b 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<sup>nd</sup> 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.

#### 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.

**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 Mouse 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.



**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-Mouse IgG2b:HRP Conjugate** into each well.

7.  Cover plate and **incubate** for  at Room Temperature.

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

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.

**Typical Example of a Work List**

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Results
1A 2A	0 Standard 0 Standard			
1B 2B	15 ng/ml 15 ng/ml			
1C 2C	30 ng/ml 30 ng/ml			
1D 2D	60 ng/ml 60 ng/ml			
1E 2E	90 ng/ml 90 ng/ml			
1F 2F	180 ng/ml 180 ng/ml			
1G 2G	360 ng/ml 360 ng/ml			
1H 2H	720 ng/ml 720 ng/ml			
3A 4A	<i>Sample</i>			
3B 4B	<i>Sample</i>			

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