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**User's Manual**

# Mouse Anti-SARS-CoV-2 IgG2a ELISA Kit

REF **DEIASL241**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

This product is used for in vitro quantitative detection of anti-SARS-CoV-2 IgG2a antibodies in mouse serum, plasma and other culture samples. It can help assess the amount of IgG2a antibodies produced by mouse after immunization or mouse IgG2a antibodies expressed in cell culture.

## Principles of Testing

This ELISA is designed, develped and produced for quantitative measurement of mouse anti-SARS-CoV-2 IgG2a in test sample. The assay utilizes the indirect enzyme linked immunoabsorbent technique with selected SARS-CoV-2 capsid core protein antigen and HRP labeled mouse IgG2a detection antibody. Assay standards and test samples are added to microtiter wells of a microplate which is coated with a highly purified SARS-CoV-2 capsid core protein antigen. After the first incubation period, the antigen on the wall of microtiter well absorbs or captures mouse anti-SARS-CoV-2 IgG2a in the sample and unbound proteins in each microtiter well are washed away. Then a HRP conjugated anti-mouse IgG2a antibody is added to each microtiter well and a link of "core antigen-mouse IgG2a-HRP conjugated detection antibody" is formed. The unbound detection antibody is removed in the sunsequent washing step. HRP conjugated detection antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the detection antibody bound to the mouse IgG2a on the wall of the microtiter well is directly proportional to the amount of antibody in the sample. A standard curve is generated by plotting the absorbance versus the respective antibody concentration for each standard. The concentration of mouse anti-SARS-CoV-2 IgG2a autoantibody in test samples is determined directly from this standard curve.

## Reagents And Materials Provided

1. SARS-CoV-2 Core Antigen Coated Microplate: 12x8 wells
2. Mouse anti-SARS-CoV-2 IgG2a Standards: 100 ng
3. Standard/Sample Diluent: 24 mL
4. HRP conjugated anti-mouse IgG1 Detection Antibody: 130 ul
5. Detection Antibody Diluent: 12 mL
6. TMB Substrate Solution: 12 mL
7. Stop Solution: 8 mL
8. Wash Buffer Concentrate (20x): 50 mL
9. Cover Film: 2 pcs
10. Sealed bag: 2 pcs
11. Instruction: 1

## Materials Required But Not Supplied

1. Precision single channel pipettes capable of delivering 10 µL, 100 µL, and 1000 µL.

2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

## Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

## Specimen Collection And Preparation

Only 1 µL of mouse serum or plasma is required for mouse anti-SARS-CoV-2 IgG2a measurement. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at –20°C or below until measurement.

Serum, plasma and other cultured samples can be stored at 2-8°C within 48 hours after specimen collection. If the experiment cannot be performed immediately, it should be stored at a temperature below -20°C for six months. The preservative sodium azide must not be added to the sample.

## Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.
2. Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 ml of Wash Buffer Concentrate (20x) into deionized or distilled water to prepare 1000 ml of Wash Buffer (1x).
3. HRP-conjugated Detection Antibody (1x) - Centrifuge the vial before opening. HRP- conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP- conjugate + 990 µl of Detection Antibody Diluent.
4. Standard - Add 1 mL of the Standard/Sample diluent to the lyophilized standard vial and mix thoroughly to prepare a 100 ng/mL standard. Please aliquot the dissolved standard and store it at -80°C to avoid repeated freezing and thawing.

Please prepare 8 tubes labeled as A-H and use the diluted standard to produce a double dilution series according to the picture shown below.

| Standard Number (Tube) | Dilutions | Total Volume (μL) | Final Concentration (ng/mL) |
|---|---|---|---|
| A | 30 μL 100ng/mL Standard + 970 μL Standard Diluent | 1000 | 3 |
| B | 300 μL Standard A + 300 μL Standard Diluent | 600 | 1.5 |
| C | 300 μL Standard B + 300 μL Standard Diluent | 600 | 0.75 |
| D | 300 μL Standard C + 300 μL Standard Diluent | 600 | 0.375 |
| E | 300 μL Standard D + 300 μL Standard Diluent | 600 | 0.1875 |
| F | 300 μL Standard E + 300 μL Standard Diluent | 600 | 0.09375 |
| G | 300 μL Standard F + 300 μL Standard Diluent | 600 | 0.046875 |
| H | 300 μL Standard Diluent | 300 | 0 |

Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 3 ng/mL, 1.5 ng/mL, 0.75 ng/mL, 0.375 ng/mL, 0.1875 ng/mL, 0.09375 ng/mL, 0.046875 ng/mL, and the last EP tubes with Standard Diluent is the blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment.

## Assay Procedure

(1) Place a sufficient number of SARS-CoV-2 antigen coated microwell strips in a holder to run mouse assay standards, controls and unknown samples in duplicate.

(2) Add 100 µL of standards into the designated microwell.

(3) Set a blank control well (when using dual-wavelength absorbance detection, it is not necessary to set a blank control well) and sample wells. For mouse serum and plasma sample, add 100 µL of sample diluent and 1 µL of serum and plasma samples into the sample wells, gently shake and mix; for cell culture samples, add 50 µL of sample diluent and 50 µL of cell culture for testing, gently shake and mix.

(4) Cover the plate with one cover film and also with sealed bag to avoid exposure to light. Incubate plate at 37°C for 60 minutes.

(5) Remove the cover film and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 300 µL of working wash solution into each well and then completely aspirating the contents. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

(6) Add 100 µL of diluted HRP conjugated anti-mouse IgG2a detection antibody working solution to each of the wells except the blank well.

(7) Cover the plate with one cover film and also with sealed bag to avoid exposure to light. Incubate plate at 37°C for 30 minutes.

(8) Repeat the wash step as described in step (5) .

(9) Add 100 µL of TMB Substrate into each of the wells.

(10) Cover the plate with one cover film and also with sealed bag to avoid exposure to light. Incubate plate at 37°C for 15 minutes.

(14) Remove the cover film and plate sealer. Add 50 µL of Stop Solution into each of the wells. Mix gently.

(16) Read the absorbance at 450 nm within 10 minutes in a microplate reader. Note: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600-630 nm.

## Quality Control

The OD value of the 10.0ng/ml standard product should be ≥0.80, the OD value of the 0ng/ml standard product should be less than 0.15, and the OD value of the blank well should be less than 0.10, then the test is valid; otherwise, it is deemed invalid and the test should be repeated.

## Calculation

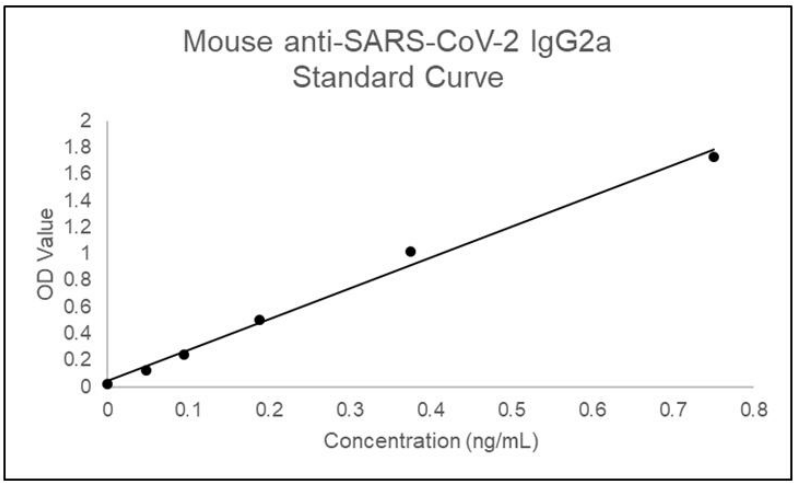
Take the concentration of the standard substance as the abscissa and the OD value as the ordinate, perform scatter plotting, select five points with better linearity, and perform linear fitting to obtain the corresponding fitting equation. Only when R2 is greater than or equal to 0.95, can it be used for calculation of antibody concentration in the sample. The target concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

When the antibody concentration in the sample is too high and exceeds the linear range, it is recommended to perform the test again after dilution to obtain an accurate quantitative concentration.

## Typical Standard Curve

A typical absorbance data and the resulting standard curve from Mosue anti-SARS-COV-2 IgG2a ELISA are represented. This curve should not be used in lieu of standard curve run with each assay.

| Concentration (ng/mL) | Absorbance Value |
|---|---|
| 0.75 | 1.7266 |
| 0.375 | 1.0177 |
| 0.1875 | 0.5017 |
| 0.09375 | 0.2414 |
| 0.046875 | 0.1199 |
| 0 | 0.0172 |




## Precision

Intra-Assay: CV<15%

Inter-Assay: CV<15%

## Detection Range

0.046875ng/mL - 3ng/mL

## Detection Limit

The minimum detectable concentration of this product for total antibodies is not higher than 20 pg/mL.

## Precautions

1. The reagents must be used in research laboratory and are for research use only.
2. Wear gloves while performing this assay and handle these reagents as if they are potential infectious.
3. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin.
4. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon contact, flush with copious amounts of water for at least 15 minutes.
5. Use Good Laboratory Practices.
6. After opening and before using, keep plate dry.
7. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
8. Storage TMB reagents avoid light.
9. Washing process is very important, not fully wash easily cause a false positive and high background.
10. Duplicate well assay is recommended for both standard and sample testing.
11. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
12. Don't reuse tips and tubes to avoid cross contamination.
13. Avoid using the reagents from different batches together.
14. The interpretation of the results of the microplate reader is completed within 30 minutes after the termination of the reaction.

## Limitations

1. This product is only used for the test of serum, plasma, cell culture and other samples. Other samples need to be evaluated before use.
2. This kit has a certain linear detection range. When the concentration of IgG2a in the sample exceeds the linear range of the kit, it will cause concentration deviation. It is recommended to dilute samples before testing for accurate evaluation of samples antibody concentration.