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User's Manual

Human Anti-Varicella Zoster Virus (gE) Antibody IgG ELISA Kit

REF **DEIA-JY2141**

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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

The ELISA kit is developed for titer measurement of Anti-Varicella Zoster Virus Antibody IgG in human serum.

General Description

Glycoprotein E (gE) is one of the known glycoproteins (gB, gC, gE, gH, gI, gK, gL) of VZV that is most abundantly expressed on the surface of virus and infected cells, playing an important role in viral replication and cell-to-cell spread. The strongly immunogenic gE can provide strong IgG signal in body fluid, which makes it ideal to be developed as an antigen for analysis of Immunogenicity in the development of VZV vaccine. Therefore, It's helpful to develop the Human Anti-Varicella Zoster Virus Antibody IgG Titer ELISA Kit (gE) to detection the IgG (gE) titer level in human serum during the clinical stage of vaccine development.

This assay kit utilizes an indirect ELISA method to measure the titer of Anti-Varicella Zoster Virus Antibody IgG. The microplate is first immobilized with Glycoprotein E (VZV), followed by the addition of samples which are then incubated and the wells washed. Secondary antibody HRP-Anti-Human IgG is then added to the plate and incubated before washing the wells again. Substrate is then loaded into the wells and the color development is monitored in proportion to the amount of antibody present. The reaction can be stopped by adding a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm, with the OD value reflecting the amount of antibody bound.

Reagents And Materials Provided

1. Pre-coated Glycoprotein E (VZV) Microplate, 1 plate, 2-8°C
2. Positive Control, 100 µL, Liquid, 2-8°C
3. Negative Control, 100 µL, Liquid, 2-8°C
4. HRP-Anti-Human IgG, 50 µL, Liquid, 2-8°C, avoid light
5. 10× Washing Buffer, 50 mL, Liquid, 2-8°C
6. Dilution Buffer, 50 mL, Liquid, 2-8°C
7. Substrate Solution, 12 mL, Liquid, 2-8°C, avoid light
8. Stop Solution, 7 mL, Liquid, 2-8°C

Materials Required But Not Supplied

1. Single or dual wavelength microplate reader with 450 nm and 630 nm filter
2. Centrifuge
3. 37 °C Incubator
4. Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision
5. 10 µL, 200 µL and 1000 µL pipette tips
6. Test Tubes

7. Graduated cylinder
8. Deionized or distilled water for dilution

Storage

The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C. The shelf life is 30 days from the date of opening.

Assay Procedure

Bring all reagents and samples to room temperature (20°C-25°C) before use.

1. Working fluid preparation

a. Preparation of 1× Washing Buffer:

Dilute 50 mL 10× Washing Buffer with ultrapure water/deionized water to 500 mL.

b. Preparation of Positive Control and Negative Control working fluid and pre-treatment of samples:

For qualitative detection of antibodies:

Dilute the Samples, Positive Control and Negative Control at 1:100 with Dilution Buffer.

For determination of antibody titer:

It is recommended to dilute the Samples, Positive Control and Negative Control from 1:100-1:51200 with Dilution Buffer.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated Glycoprotein E (VZV) Microplate. Each experiment requires a set of Positive Control and Negative Control working fluid.

3. Add Samples

Add 100 µL diluted samples, Positive Control and Negative Control working fluid to the corresponding wells. Add 100 µL Dilution Buffer to blank control. Seal the plate with microplate sealing film and incubate at 37°C for 1.0 h.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1× Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1× Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

5. Add HRP-Anti-Human IgG

Dilute HRP-Anti-Human IgG solution at 1:2000 with Dilution Buffer to make a working solution. The prepared working fluid should be stored away from light. For all wells, add 100 µL HRP-Anti-Human IgG working solution. Seal the plate with microplate sealing film and incubate at 37°C for 1.0 h, avoid light.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 min, avoid light.

8. Termination

Add 50 µL Stop Solution to each well, and tap the plate gently for 3 min to allow thorough mixing.

Note: the color in the wells should change from blue to yellow.

9. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer. **Note:** To reduce the background noise, subtract the value read at $OD_{450\ nm}$ with the value read at $OD_{630\ nm}$.

Interpretation Of Results

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1

Normal range of Negative control (1:100): $OD_{450\ nm}$-$OD_{630\ nm}$ < 0.1

Normal range of Positive control (1:1600): $OD_{450\ nm}$-$OD_{630\ nm}$ ≥ 1.5

Note:The cut-off value can be determined by the end user.

a. For qualitative detection of antibodies:

Positive reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ≥ Cut-off value means Anti-Varicella Zoster Virus Antibody IgG are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample < Cut-off value means Anti-Varicella Zoster Virus Antibody IgG are not detected.

b. For determination of antibody titer:

Determination of antibody titer: the positive sample was diluted with a gradient, and the antibody titer of the sample corresponds to the highest dilution factor that still yields a positive reading.

Typical Standard Curve

For qualitative detection of antibodies:

Value Result in units	Result	Test Result Interpretation
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.065	Negative	Anti-Varicella Zoster Virus Antibody IgG are not detected
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.472	Positive	Anti-Varicella Zoster Virus Antibody IgG are detected

For determination of antibody titer:

Quality control data between dif erent plates should not be mixed, and negative and positive controls should be set for each test.

Ratio of Dilution	$OD_{450\ nm}$ - $OD_{630\ nm}$ (**Samples**)	Result
100	3.296	The titer level of antibody is 25600
200	3.173	
400	2.979	
800	2.730	
1600	1.982	
3200	1.196	
6400	0.673	
12800	0.369	
25600	**0.180**	
51200	0.087	
Blank	0.012	

Precautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
5. This kit should be stored at 2°C -8°C.
6. Please prepare the working solution of each component according to the needs of the experiment. Except for 10× Washing Buffer, all prepared working solution is for one-time use and cannot be stored.

Limitations

The kit cannot be used for quantitative detection.