Version 22-04/24

User's Manual

Varicella Zoster Virus Glycoprotein E (VZV gE) ELISA Kit

REF **DEIA-NS2307-3**

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RUO

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 kit is developed for quantitative detection of Varicella Zoster Virus Glycoprotein E (VZV gE) in vaccine samples. It is intended for research use only (RUO).

General Description

Varicella-zoster virus (VZV) the etiologic agent of chickenpox and herpes zoster [HZ], is highly contagious and still endemic worldwide. Glycoprotein E (gE) is one of the known glycoproteins (gB, gC, gE, gH, gI, gK, gI) 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,Its helpful to develop the Varicella Zoster Virus Glycoprotein E (VZV gE) ELISA Kit to quantitative detection the VZV gE antigen in vaccine samples during the manufacture and quality control of vaccine development.

Principles of Testing

This assay kit is used to measure the levels of Glycoprotein E (VZV) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-Glycoprotein E (VZV) Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin- Anti-Glycoprotein E (VZV) Antibody to the plate, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of Glycoprotein E (VZV) present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of Glycoprotein E (VZV) bound.

Reagents And Materials Provided

1. Pre-coated Anti-Glycoprotein E (VZV) Antibody Microplate, 1 plate, Solid. Unopened 2-8°C, Opened 2-8°C.
2. Glycoprotein E (VZV) Standard, 30 µg, Powder. Unopened 2-8°C, Opened -70°C.
3. Biotin-Anti-Glycoprotein E (VZV) Antibody, 20 µg, Powder. Unopened 2-8°C, Opened -70°C.
4. Streptavidin-HRP, 50 µL, Liquid. Unopened 2-8°C, avoid light. Opened 2-8°C, avoid light.
5. 10× Washing Buffer, 50 mL, Liquid. Unopened 2-8°C. Opened 2-8°C.
6. 2×Dilution Buffer, 50 mL, Liquid. Unopened 2-8°C, Opened 2-8°C.
7. Substrate Solution, 12 mL, Liquid. Unopened 2-8°C, avoid light. Opened 2-8°C, avoid light.
8. Stop Solution, 7 mL, Liquid, Unopened 2-8°C, Opened 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. 10 µL, 200 µL and 1000 µL precision pipettes
5. 10 µL, 200 µL and 1000 µL pipette tips
6. Multichannel pipettes
7. Tubes
8. Graduated cylinder to prepare Wash Solution
9. Deionized or distilled water to dilute 10× Washing Buffer

Storage

1. The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per "Reagents And Materials Provided". The shelf life is 30 days from the date of opening.

Note: Do not use reagents past their expiration date. Find the expiration date on the outside packaging.

Reagent Preparation

1. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in a 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.
2. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70°C. It is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5 µg.

Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
Glycoprotein E (VZV) Standard	30 µg	200 µg/mL	150 µL water
Biotin-Anti-Glycoprotein E (VZV) Antibody	20 µg	100 µg/mL	200 µL water

Assay Procedure

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

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

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-Glycoprotein E (VZV) Antibody working fluid:

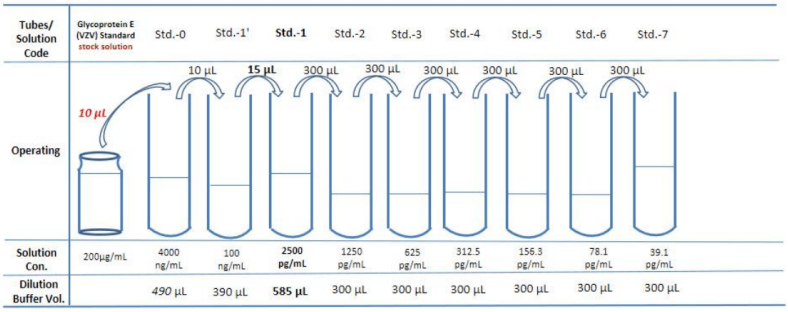
Dilute Biotin- Anti-Glycoprotein E (VZV) Antibody to 0.5 µg/mL with Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard curve

Make serial dilutions of the Glycoprotein E (VZV) as a Standard curve with Dilution Buffer as recommended in Figure.



3. Add Samples

Add 100µL serially diluted Glycoprotein E (VZV) Standard curve and samples to each well. For blank Control wells, please add 100 µL 1×Dilution Buffer. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

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 Biotin-Anti-Glycoprotein E (VZV) Antibody

For all wells, add 100 µL Biotin -Anti-Glycoprotein E (VZV) Antibody (dilute to 0.5 µg/mL) working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

6. Washing

Repeat step 4.

7. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (1:2000 dilute) working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour, avoid light.

8. Washing

Repeat step 4.

9. Substrate Reaction

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

10. Termination

Add 50 µL Stop Solution to each well, and tap the plate gently for 5 min to allow thorough mixing.

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

11. 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 OD450 nm with the value read at OD630 nm.

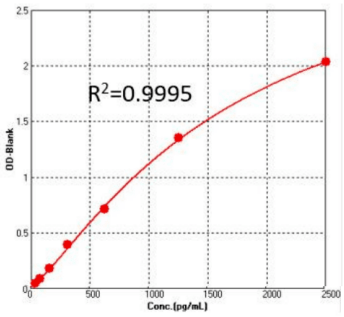
Calculation

1. Normal range of Standard curve: R2≥0.9900, detection range: 39.1-2500 pg/mL.
2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.
3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

Typical Standard Curve

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Glycoprotein E (VZV) Standard(pg/mL)	OD450-630nm	OD450-630nm-Blank
2500	2.101	2.032
1250	1.422	1.353
625	0.783	0.714
312.5	0.462	0.393
156.25	0.248	0.179
78.125	0.157	0.088
39.0625	0.115	0.046
Blank	0.069	0.000



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 1× Washing Buffer, all prepared working solution is for one-time use and cannot be stored.