A185-EN.01

# Varicella Zoster Virus Glycoprotein E (VZV gE) ELISA Kit (For Vaccine Development)

**Pack Size: 96 tests**

**Catalog Number：** RAS-A185

**IMPORTANT: Please carefully read this manual before performing your experiment.**

***For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures***

## 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).

## PRINCIPLE OF THE ASSAY

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, it's 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.

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 HRP-Anti-Glycoprotein E (VZV) Antibody 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 450nm and 630nm. The OD Value reflects the amount of Glycoprotein E (VZV) bound.



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## MATERIALS PROVIDED

**TABLE 1. MATERIALS PROVIDED**

| Catalog | Components | Size (96 tests) | Format | Storage | |
|---|---|---|---|---|---|
| | | | | Unopened | Opened |
| RAS185-C01 | Pre-coated Anti-Glycoprotein E (VZV) Antibody Microplate | 1 plate | Solid | 2-8°C | 2-8°C |
| RAS185-C02 | Glycoprotein E (VZV) Standard | 30 μg | Powder | 2-8°C | -70°C |
| RAS185-C03 | HRP-Anti-Glycoprotein E (VZV) Antibody | 15 μg | Powder | 2-8°C, avoid light | -70°C, avoid light |
| RAS185-C04 | 10xWashing Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| RAS185-C05 | 2xDilution Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| RAS185-C06 | Substrate Solution | 12 mL | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| RAS185-C07 | Stop Solution | 7 mL | Liquid | 2-8°C | 2-8°C |

## REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Centrifuge;

37° C Incubator;

10 μL, 200 μL and 1000 μL precision pipettes;

10 μL, 200 μL and 1000 μL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10×Washing Buffer;

## SHIPPING AND STORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.

2. The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

***Note:*** *a. Do not use reagents past their expiration date.*



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*b. Find the expiration date on the outside packaging.*

## REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in a 37ºC 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 2 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℃. It is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5 μg.

**TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS**

| ID | Components | Size | Stock Solution Con. | Reconstitution Buffer and Vol. |
|---|---|---|---|---|
| RAS185-C02 | Glycoprotein E (VZV) Standard | 30 μg | 200 μg/mL | 150 μL water |
| RAS185-C03 | HRP-Anti-Glycoprotein E (VZV) Antibody | 15 μg | 100 μg/mL | 150 μL water |

## RECOMMENDED SAMPLE PREPARATION

### 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 HRP-Anti-Glycoprotein E (VZV) Antibody working fluid:

Dilute HRP-Anti-Glycoprotein E (VZV) Antibody to 0.05 μg/mL 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 1.



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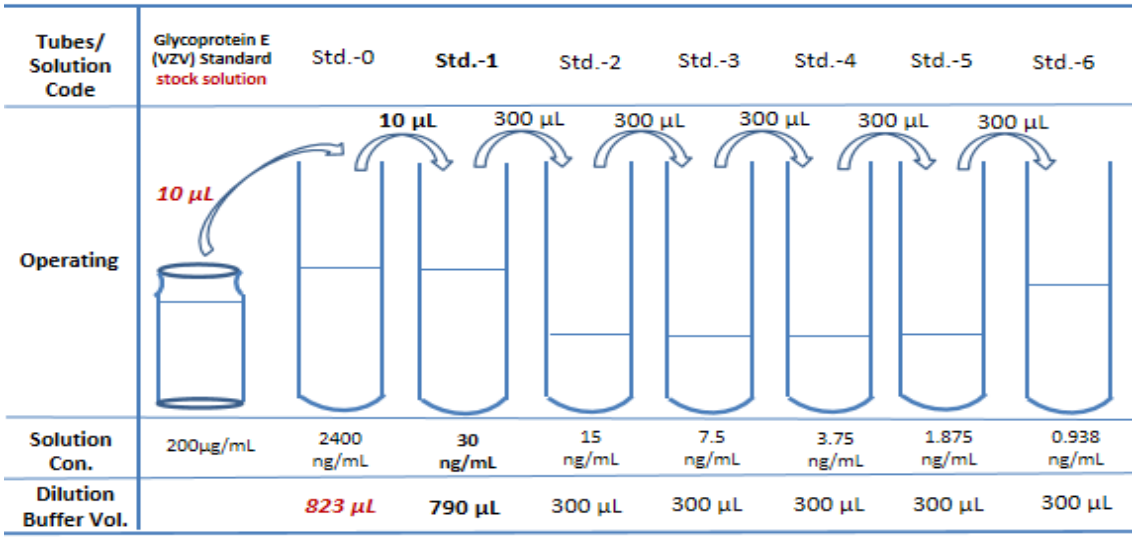
**FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Glycoprotein E (VZV)**




## 3. Add Samples

Add 100 µL 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 37℃ 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 HRP-Anti-Glycoprotein E (VZV) Antibody

For all wells, add 100 µL **HRP-Anti-Glycoprotein E (VZV) Antibody (dilute to 0.05 µg/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour.

## 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℃ for 20



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min, avoid light.

## 8. 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.*

## 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* $OD_{630\ nm}$.

## CALCULATION OF RESULTS

1. Normal range of Standard curve: $R^2 \geq 0.9900$, detection range: 0.938-30 ng/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 from 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. Linear fittin are used to draw the standard curve and calculate the sample concentration.

## PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C.

## TYPICAL DATA

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



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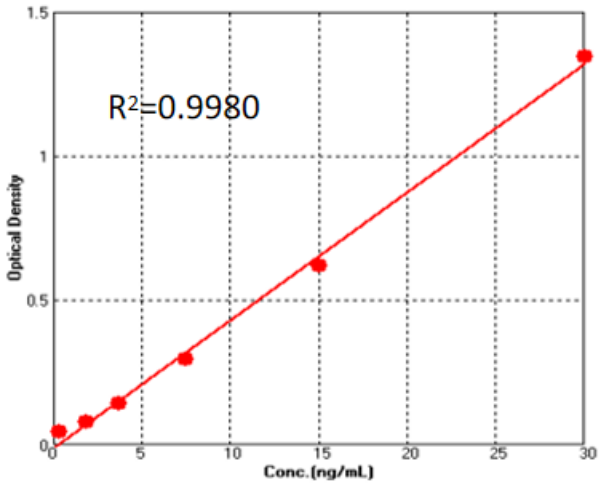
| Glycoprotein E (VZV) Standard(ng/mL) | OD450-630nm | OD450-630nm-Blank |
|---|---|---|
| 30 | 1.344 | 1.328 |
| 15 | 0.620 | 0.605 |
| 7.5 | 0.298 | 0.283 |
| 3.75 | 0.144 | 0.128 |
| 1.875 | 0.078 | 0.062 |
| 0.938 | 0.046 | 0.030 |
| Blank | 0.016 | 0.000 |



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