

Varicella Zoster Virus Glycoprotein E (VZV gE) ELISA Kit

Pack Size: 96 tests

Catalog Number: RAS-A102

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, 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 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 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.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS102-C01	Pre-coated Anti-Glycoprotein E (VZV) Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RAS102-C02	Glycoprotein E (VZV) Standard	30 µg	Powder	2-8°C	-70°C
RAS102-C03	Biotin-Anti-Glycoprotein E (VZV) Antibody	20 µg	Powder	2-8°C	-70°C
RAS102-C04	Streptavidin-HRP	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS102-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS102-C06	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS102-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS102-C08	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. 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 TABLE 1. The shelf life is 30 days from the date of opening.

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

b. 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 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°C. 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.
RAS102-C02	Glycoprotein E (VZV) Standard	30 µg	200 µg/mL	150 µL water
RAS102-C03	Biotin-Anti-Glycoprotein E (VZV) Antibody	20 µg	100 µg/mL	200 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 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:

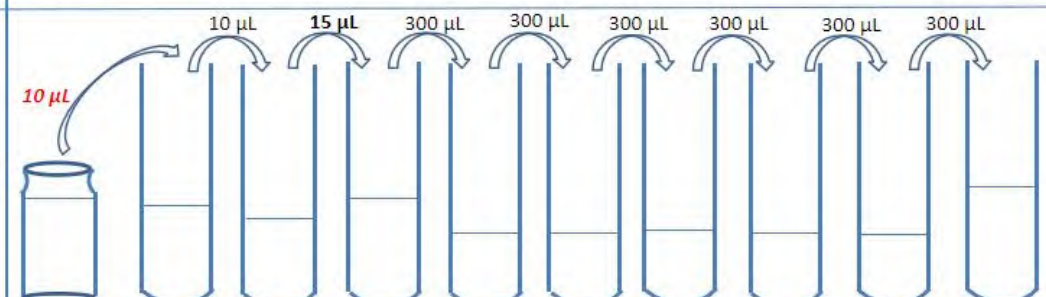
Dilute Streptavidin-HRP at 1:2000 with Dilution Buffer. The prepared working fluid should avoid light. Please prepare

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 1.

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

Tubes/ Solution Code	Glycoprotein E (VZV) Standard stock solution	Std.-0	Std.-1'	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6	Std.-7
Operating		10 µL	15 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL
Solution Con.	200 µg/mL	4000 ng/mL	100 ng/mL	2500 pg/mL	1250 pg/mL	625 pg/mL	312.5 pg/mL	156.3 pg/mL	78.1 pg/mL	39.1 pg/mL
Dilution Buffer Vol.		490 µL	390 µL	585 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL

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 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 $OD_{450\text{ nm}}$ with the value read at $OD_{630\text{ nm}}$.

CALCULATION OF RESULTS

1. Normal range of Standard curve: $R^2 \geq 0.9900$, 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.

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

TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only.

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

