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Varicella zoster virus(VZV) Recombinant gE protein Quantitation Kit (ELISA)

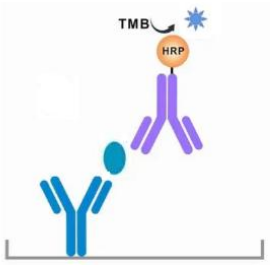
Cat#17-0123

This product is used to detect the content of recombinant gE protein in samples

For research use only, not for diagnostic use.

1. Experimental Principle

This product adopts the principle of double antibody sandwich method. A recombinant gE protein monoclonal antibody is coated with an enzyme-linked reaction plate, and the samples to be tested are added for reaction. After washing, the unbound compounds are removed, and another recombinant gE protein monoclonal antibody labeled by HRP is added to form an antibody-antigen-antibody-HRP complex. The content of rashes recombinant gE protein in the sample to be tested was indicated by TMB chromogenic degree.



2. Use Instructions

Please read this instruction carefully prior to beginning the assay.

- Observe good laboratory practices：Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

3. Materials and Reagents Provided

Item	Quantity	Storage Condition
Pre-Coated 96-Well Microplate	8 Wells×12 Strips	2~8℃
HRP Conjugate (500×)	50μL	-15 ~ -25℃
TMB Substrate II	11mL	2~8℃
Casein-Na	0.5g	2~8℃
HRP Conjugate Dilution	12mL	2~8℃
20×PBST	50mL	2~8℃
Plate Seal	2	2~8℃/Room temperature
Instructions	1	2~8℃/Room temperature

4. Materials Required, Not Supplied

These materials are not included in the kit, but are used in the testing process:

- Herpes zoster recombinant gE Protein reference (available from ABmax)
- Enzyme marker, deionized water, multi-channel and single-channel pipettes, dilution tubes, shock incubators.
- Stop Solution

Preparation methods：Measure 500mL of purified water into the container, measure 108.7mL of concentrated sulfuric acid, slowly pour into the purified water along the wall of the device, stirring without stopping, and then measure 200mL of purified water to rinse the measuring cup of concentrated sulfuric acid, slowly pour the rinsing solution into the sulfuric acid solution, wash it again with 100mL of purified water, and pour the rinsing solution into the acid solution. Finally, add purified water to the final volume of 1000mL.

5. Storage conditions and Expiration date

- HRP Conjugate (500×) stored at -15 ~ -25℃, other components stored at 2~8℃ protected from light.
- Period of validity is 12 months.

6. Operation Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- All samples should be mixed thoroughly and gently.
- Incubate ELISA plates on a plate shaker during all incubation steps.

7. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25℃) prior to use.
- 20×PBST may contain precipitate, this is normal.If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

7.1 1×PBST

Take one bottle of 20×PBST, dilute to 1000 mL with deionized water, mix and backup.

The amount of liquid can be adjusted according to the experimental needs and current distribution better.

7.2 0.5%Casein-Na

Accurately weigh 0.5g Casien-Na and completely dissolve into 100mL1×PBST, mix thoroughly and use as a 0.5% Casein-Na Buffer. The amount of liquid can be adjusted according to the experimental needs and current distribution better.

7.3 Preparation of Enzyme Conjugates

Calculate the volume of working solution required for the experiment, and take an appropriate amount of Enzyme Conjugate (500×）Dilute with HRP Conjugate Dilution at 100 times, then dilute again at 5 times, mix well and set aside.

8. Preparation of reference materials and samples

- Use 0.5%Casein-Na diluent to prepare reference products of different concentrations (2x gradient dilution) : 3200ng/mL, 1600ng/mL, 800ng/mL, 400ng/mL, 200ng/mL, 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.125ng/mL, 0ng/mL (0.5%Casein -Na diluent)
- Use 0.5%Casein-Na diluent to dilute the samples to the standard curve range for testing. If the concentration of the samples to be tested is unknown, the samples can be tested by gradient dilution of 10 times, 30 times, 100 times, 300 times and 1000 times, and then the measured value (ng/mL) is calculated by selecting the OD value within the standard curve range. The final sample concentration should be multiplied by the sample dilution factor on the basis of the measured value.
- New reference materials and samples should be prepared before each use, and discarded after use.

9. Operating Rules

- Prepare all reagents, references, and samples as required in the previous sections.
- This microplate is readily available and there is no need to rinse before use.

9.1 Add Samples

All the reference products and samples were added to the enzyme label plate, marked well, and samples were added to each well by 100μL (with multiple Wells). After sealing the plate, a constant temperature shock incubator was placed at 37℃ at 300rpm and incubated for 30 minutes.

9.2 Washing

Discard the liquid in each hole, add 350μL 1×PBST to each hole, leave for 30 seconds, discard the liquid in the hole, repeat 3 times, and pat dry on a kleentowel after the last wash.

9.3 Add Enzyme Conjugates

Add 100μL working liquid for antibody detection to each well, seal the plate with a new sealing plate film and put it in a constant temperature shock incubator at 37℃ at 300rpm for 30 minutes.

9.4 washing

Repeat Step 9.2.

9.5 Add TMB Substrate

Add TMB Substrate II 100 μL/well . Shake slightly and mix well.

9.6 Stop

Add termination solution 50μL to each well and mix slightly.

9.7 Reading

Read absorbance at 450/630nm(OD=OD_{450}-OD_{630}).

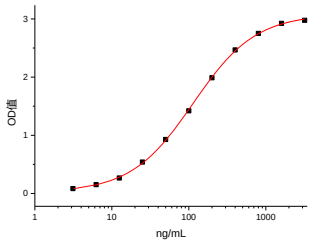
10. Result Processing

10.1 standard curve

Calculate the OD mean of the reference (see the following example, for example only, based on the actual measurement) :

gE protein standard, ， ng/mL	OD	OD	Mean
3200	2.996	2.958	2.977
1600	2.931	2.917	2.924
800	2.701	2.800	2.751
400	2.416	2.517	2.467
200	2.009	1.969	1.989
100	1.414	1.426	1.420
50	0.936	0.919	0.928
25	0.523	0.557	0.540
12.5	0.265	0.267	0.266
6.25	0.151	0.150	0.151
3.125	0.082	0.083	0.083
NC	0.012	0.011	0.012

The standard concentration and the corresponding OD mean value are then fitted in and calculated to obtain the standard curve.



The Standard Curve（R^2=0.99979）

10.2 Calculation of the Sample Concentration

Select the OD value within the range of the standard curve to calculate the sample concentration, that is, the measured value of the sample (ng/mL), and multiply the measured value by the dilution multiple of the sample to obtain

the sample concentration.

11. Product Performance Index

- Linearity range：3.125～400ng/mL。
- Sensitivity：（NC+3SD）：1.0ng/mL。
- Accuracy and Precision: The recovery rate of three quality control points (High, medium, and low concentrations) are within the range of 80%~120%,the ccuracy can mee the requirements; The CV (%) is 10%, the Precision can mee the requirements.

Accuracy and Precision	Accuracy（the recovery rate%）			Precision（CV%）		
Runs	Run1	Run2	Run3	Run1	Run2	Run3
HQC-300ng/mL	102%	91%	97%	2.24%	3.75%	0.34%
MQC-75ng/mL	96%	102%	94%	1.11%	3.50%	0.84%
LQC-15ng/mL	103%	86%	105%	4.71%	2.68%	4.80%

- HOOK Effect:：When using a reference test higher than 3200ng/mL, the signal value did not decrease and there was no HOOK effect.
- Dilution linearity: Select reference products higher than 3200ng/mL and dilute them to the standard curve range (300ng/mL, 75ng/mL, 15ng/mL), with calculation accuracy and deviation within the range of 80% ~ 120%.

12. Problems and Solutions

Problems	Reason	Solutions
The standard curve is poor	The flank was improperly diluted	Check the straw, use the corrected pipette, operate properly, and re-dilute.
The signal value is low	The incubation time was short	Ensure adequate incubation time.
	Insufficient volume or improper dilution of the reagents	Check the pipette to ensure that the reagent is properly and adequately prepared.
	Long-term exposure of the chromogen solution	The color rendering solution should be protected from light and replaced with a new color rendering solution.
The CV value is larger	Insufficient washing	Follow the washing procedure strictly operated.
	The detergent was contaminated	Replace the new lotion.
Lack of sensitivity	Improper storage of the ELISA kit	Keep it according to the instructions.
There was a crystallization in the dilution solution	Precipitation and / or solidification of the components in the diluent.	The diluent was heated to 37 C to completely dissolve the precipitate.