Version 22-04/24

User's Manual

Mouse Anti-Varicella Zoster Virus (VZV/chickenpox) IgM ELISA kit

REF **DEIA-NS2307-8**

Σ **96T**

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

CD's Varicella Zoster IgM Antibody ELISA Test Kit has been designed for the detection of IgM class antibodies against Varicella Zoster in mouse serum and plasma (citrate, heparin). This kit is for in vitro research use only.

General Description

Varicella-zoster virus (VZV) is known by many names, including chickenpox virus, varicella virus, zoster virus, and human herpes virus type 3 (HHV-3). VZV is closely related to the herpes simplex viruses (HSV), sharing much genome homology. It is one of eight herpes viruses known to infect humans and other vertebrates. It commonly causes chickenpox in children and adults and herpes zoster (shingles) in adults and rarely in children. As with the other herpes viruses, VZV causes both acute illness and lifelong latency. Before vaccination became widespread, acute primary infection (varicella or"chickenpox") was common during childhood--especially in temperate climates. Varicella usually is a benign and self-limiting illness but can be more severe in adults and in individuals with cellular immunodeficiency. These individuals are at much higher risk of pneumonia and disseminated disease with visceral involvement. Zoster typically presents as a painful, localized cutaneous eruption occurring along 1 or more contiguous dermatomes. Humans are the only known natural hosts of VZV. Transmission of VZV occurs through direct contact with infectious lesions or by inoculation of aerosolized infected droplets onto a susceptible mucosal surface.

The known envelope glycoproteins (gB, gC, gE, gH, gI, gK, gL) correspond with those in HSV; however, there is no equivalent of HSV gD. The most popular test detects VZVspecific IgM antibody in blood; this appears only during chickenpox or herpes zoster and not while the virus is dormant.

Principles of Testing

The Varicella zoster IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Varicella zoster antigens are bound on the surface of the microtiter strips. Diluted unknowns are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Varicella zoster antigen takes place. After one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-mouse-IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 15 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color.

Reagents And Materials Provided

1. Varicella zoster antigen coated strip plate, (8×12 strip or 96 wells), 1 plate
2. Calibrator A (Negative Control); 2.0 mL; 1 vial
3. Calibrator B (Cut-off Control); 3.0 mL; 1 vial

4. Calibrator C (Positive control); 2.0 mL; 1 vial
5. Anti-Mouse IgM-HRP Conjugate, (20 ml), 1 bottle
6. Sample Diluent, 100 ml, 1 bottle
7. Wash buffer (20×), 50 ml, 1 bottle
8. TMB Substrate Solution, 15 ml, 1 bottle
9. Stop Solution, 15 ml, 1 bottle

Materials Required But Not Supplied

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

Storage

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots and should be stable for 3 months.

Specimen Collection And Preparation

Principally serum or plasma (Citrate, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Heat inactivation of samples is not recommended.

For the performance of the test the samples (not the standards) must be diluted 1:100 with ready-to-use sample diluent (e.g., 5 µL serum + 500 µL sample diluent and thoroughly mix with a vortex). Do not dilute the calibrators.

Vaccinated mouse samples

Athough we recommend testing of mouse samples at 1:100, but other sample dilutions may be considered from 1:20 or higher to distinguish between the normal and vaccinated animals.

Reagent Preparation

1. Dilute Wash buffer (20×) 1:19 with distilled water. (e. g. 10 mL Washing Buffer + 190 mL distilled water.) Store diluted buffer at 4°C for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes. **All reagents must be at room temperature prior to their use.**

Assay Procedure

(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE)

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute all samples 1:100 with the sample diluent. It is recommended to prepare a parallel replica plate containing all sample for quick transfer to the coated plate. DO NOT dilute calibrators or controls. Dilute wash buffer stock (20×) 1:19 with distilled water.

1. Label or mark the microtiter well strips to be used on the plate
2. Dispense 100 ul diluent in 1 well to be used as blank. Pipet 100 µl of calibrators, controls, and diluted (1:100) samples into appropriate wells in duplicate. Cover the plate, mix gently for 5-seconds and incubate at 37°C for 60 min.
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, wash the wells 3 times with 300 ul of 1× wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add 100 µl anti-mouse IgM-HRP conjugate to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for 30 minutes at room temp (20-25°C).
5. Wash the wells 3 times as in step 3.
6. Add 100 µl TMB substrate solution. Mix gently for 5-10 seconds. Cover the plate and incubate for 15 minutes at room temp (20-25°C) in the dark (the incubation time may be varied by 5-10 min so as to to get the maximum A450 of no greater than 2.5). Blue color develops in positive controls and samples.
7. Stop the reaction by adding 100 ul of stop solution to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).
8. Measure the absorbance at 450 nm and 630nm as reference using an ELISA reader within 30 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Do not touch the bottom of the wells.

Calculation

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.

	OD
Calibrator A (Negative Control)	0.012
Calibrator B (Cut off Control)	0.479
Calibrator C (Positive Control)	1.147

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards. The OD of the calibrators (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log. The initial dilution of unknowns has been taken

into consideration when reading the results from the graph. Results of unknowns of higher predilution must be adjusted for the dilution factor. Unknowns showing concentrations above the highest calibrator must be diluted as described in "Assay Procedure" and reassayed.

Run Validation Criteria

For an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

1. Substrate Blank: Absorbance value < 0.100
2. Negative Control: Absorbance value < 0.200 and < Cut-off
3. Cut-off Control: Absorbance value 0.150 – 1.300
4. Positive Control: Absorbance value > Cut-off

If these criteria are not met, the test is not valid and must be repeated.

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control

0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

Results in Units [U]

Sample (mean) absorbance value × 10 / Cut-off = [Units = U]

Example: 1.591 × 10 / 0.43 = 37 U (Units)

Interpretation Of Results

There are no approved values for mouse samples so the recommended values for the positive samples are based upon human sample guidelines.

Sample Values:

U	Interpretation
<9	**negative**
9-11	**equivocal**
>11	**positive**
10	**Cut-Off**

In an in-house study apparently healthy research subjects showed the following results:

Ig isotype	n	Interpretations		
		positive	**equivocal**	**negative**
IgM	56	85.7%	3.6%	10.7%

Normal mouse samples:

Normal mouse samples (non-vaccinated samples) yielded values below the cut-off when tested at 1:100.

Precision

Intraassay: <12.49%

Interassay: <10.02%

Specificity

This kit detects IgM subtype of mouse anti-VZV with no significant reactivity with the mouse IgA or IgG. This kit is not designed for other species. ADI has separate human anti-VZV Ig ELISA kits.

Precautions

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking, and smoking in the lab must be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless, precautions like the use of latex gloves must be taken. Serum and reagent spills must be wiped off with a disinfecting solution (e.g., sodium hypochlorite, 5%) and must be disposed of properly. All reagents must be brought to room temperature (18 to 25°C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care must be taken that the stoppers are not contaminated. Further a possible mix-up must be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots must be used, they should not be mixed among one another. All reagents must be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa must be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

Limitations

This assay is intended for research use only – not for use in diagnostic procedures. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless, precautions like the use of latex gloves must be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g., sodium hypochlorite, 5%) and have to be disposed of properly. All reagents must be brought to room temperature (20 to 25 °C) before performing the test.

Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.

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