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User's Manual

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

REF **DEIA-NS2307-9**

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 monkey (rhesus, cynomolgous, and baboon) serum or plasma (Citrate, heparin). This kit is for in-vitro research use only (RUO) and not for diagnosis cure or prevention of the disease.

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 VZV specific IgM antibody in blood; this appears only during chickenpox or herpes zoster and not while the virus is dormant.

Varicella Virus Infection in monkey

Human VZV is specific for human host. Simian varicella virus (SVV), the etiologic agent of naturally occurring varicella in primates, is genetically and antigenically closely related to human varicella zoster virus (VZV). Experimental inoculation of mice, rats and nonhuman primates (NHP) with VZV results in seroconversion but not varicella. There is a scarcity of the human, mouse (animal) and simian antibody ELISA kits so we do not know the extent of antibody cross reactivity between human and simian VZV antigens.

Principles of Testing

The Varicella zoster IgM antibody test kit is based on the principle of the indirect ELISA. Varicella zoster antigen is 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, to remove unbound material. Then Antibody-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 mL; 1 vial
3. Calibrator B (Cut-off Control); 3 mL; 1 vial
4. Calibrator C (Positive control); 2 mL; 1 vial
5. Anti-monkey 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. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:50 with ready-to-use sample diluent (e.g. 5 µL serum + 250 µL sample diluent). Run in test in duplicate (100 ul/well). Do not dilute the calibrators.

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 1:50 with the sample diluent. It is recommended to prepare a parallel replica plates 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 µl diluent in 1 well to be used as blank. Pipet 100 µl of calibrators, controls, and diluted 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 µl 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-monkey 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. Blue color develops in positive controls and samples.
7. Stop the reaction by adding 100 µl 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 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

Run Validation Criteria:

In order 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
5. Positive Control: Absorbance value > Cut-off

CALCULATION

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

Normal Values (human)

These values are derived from human samples to be used as general guidance as there is no monkey specific data available.

Cut-off	Negative	Equivocal	Positive
10 U	<9 U	9-11 U	>11 U

Monkey Sample

Serum samples from a mixed population of adult monkeys (rhesus, cyonomolgous, and baboon) were tested at 1:100 sample dilution. Given the low basal values in most monkeys, it is possible to test the samples at 1:50 dilution to improve sensitivity.

Sample	Baboon	Rhesus	Cynomologus
1	0.430	0.021	0.160
2	0.028	0.178	0.014
3	0.018	0.016	0.014
4	0.019	0.016	0.012
5	0.058	0.041	0.013
6	0.042	0.297	0.170
7	0.142		
8	0.273		

The values are absorbance at 450nm.

Precision

Intraassay: <12.49%

Interassay: <10.02%

Specificity

Antigen Specificity and Species reactivity

VZV (Ellen) is grown in human Fibroblast cells is extracted lysed cells using proprietary methods. The antigen preparation is partially purified to reduce host cell components and contains predominantly VZV antigens. The antigen is inactivated using detergents. However, it must be treated as if infectious and properly disposed. The kit will detect antibodies to several antigenic proteins of VZV.

This kit is designed to be used in monkey samples and it has been tested with rhesus, cynomolgous and baboon samples. CD has similar kits for mouse samples for testing the efficacy of vaccines in mice and other animals.

Precautions

Only for in-vitro use! Do not ingest or swallow! All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. 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. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

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