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User's Manual

VZV IgM ELISA Kit

REF **DEIA500**

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

VZV IgM ELISA Kit is intended for in vitro diagnosis of VZV associated diseases, namely varicella and herpes zoster. The diagnostic kit can also be utilized for differential diagnosis of neuroinfections, infections of eye and skin exanthematous diseases.

General Description

Varicella zoster virus (VZV) is one of eight herpes viruses known to infect humans (and other vertebrates). It commonly causes chicken-pox in children and Herpes zoster (shingles) in adults and rarely in children.

Principles of Testing

VZV IgM ELISA Kit is a solid-phase immunoanalytical test. The polystyrene strips are coated with specific antigen that bear immunodominant epitopes of VCA complex. The antiVCA EBV antibodies, if present in the tested sera, bind to the immobilized antigens and the antibodies being in complexes with antigen are later on recognised by animal anti-human IgM antibodies labelled with horseradish peroxidase. The labelled antibodies are revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in colour, if present, may be attributed to the reaction background. Dilution buffer "plus" contains RF sorbent to saturate IgG and rheumatoid factor (RF). It is possible to prepare the "plus" buffer by mixing with Dilution buffer r.t.u. and RF sorbent according to the Instruction manual.

Reagents And Materials Provided

1. **ELISA break-away strips (colourless) coated with specific antigens**: 1 microplate
2. 1.3 mL **Positive control serum1**, ready to use: 1 vial
3. 1.3 mL **Negative control serum**, ready to use: 1 vial
4. 1.3 mL **Calibrator**, ready to use: 1 vial
5. 1 mL **RF sorbent2, 51x concentrated**: 1 vial
6. 0.2 mL **Anti-human IgG antibodies labelled with horseradish peroxidase 101x concentrated (Px-conjugate)**: 1 vial
7. 125 mL **Wash buffer concentrate, 10x concentrated**: 1 vial
8. 125 mL **Dilution buffer (DB)**, ready to use: 1 vial
9. 15 mL **Chromogenic substrate (TMB substrate)**, ready to use: 1 vial
10. 30 mL **Stop solution**, ready to use: 1 vial
11. Sealable pouch for unused strips
12. Instruction manual
13. Certificate of quality

Materials Required But Not Supplied

1. Distilled or deionised water for dilution of the Wash buffer concentrate.
2. Appropriate equipment for pipetting, liquid dispensing and washing.
3. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

Storage

1. Store the kit reagents at +2 to +10°C, in a dry place and protected from the light. Store unused strips in the sealable pouch and keep the desiccant inside.
2. Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.
3. Do not store diluted samples and a diluted Px-conjugate. Always prepare fresh.
4. Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.
5. Expiration date is indicated at the ELISA kit label and at all reagent labels.

Reagent Preparation

1. Allow all kit components to reach room temperature.
2. Vortex samples (sera), Calibrator and Control sera in order to ensure homogeneity and mix all solutions well prior use.
3. Prepare Dilution buffer Plus: dilute RF sorbent 1: 50 by Dilution buffer (i.e. 0.1 mL RF sorbent + 5 mL Dilution buffer. (Prepare only an amount necessary for the run, do not store)
4. Dilute serum samples 1:100 in Dilution buffer Plus and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). Mix carefully and incubate 10 min. at room temterature. Do not dilute the Calibrator, Positive and Negative control serum, they are ready to use.
5. Prepare Wash buffer by diluting the Wash buffer concentrate 10x with an appropriate volume of distilled or deionised water (e.g. 100 mL of the concentrated Wash buffer +900 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one week if stored at +2 to +10°C.
6. Dilute the concentrated Px-conjugate 1:100 with Dilution buffer (e.g. 0.1 mL Pxconjugate + 10 mL Dilution buffer). (Note: For one microtitre plate you will need approx. 12 mL of the diluted Px-conjugate., i.e. 0,12 mL of the concentrated Pxconjugate + 12 mL of Dilution buffer).
7. Do not dilute TMB substrate and Stop solution, they are ready to use.

Assay Procedure

1. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
2. Pipette Calibrator, Controls and samples according to the pipetting scheme. Start with filling the first well with 100 µl of Dilution buffer Plus (DB-plus) to estimate the reaction background. Fill the next wells with 100 µl of Negative and Positive control sera, Calibrator apply into triplet of the wells. Fill the remaining wells with 100 µl of serum samples. Opalescence in diluted samples does not interfere in the test performance. It is

sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the samples in doublets. Incubate 60 minutes (±5 min) at room temperature.

3. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant. Wash and aspirate the wells four times with 250 µl/well of Wash buffer. Avoid crosscontamination between wells! If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.
4. Add 100 µL of diluted Px-conjugate into each well.
5. Incubate 60 minutes (±5 min) at room temperature.
6. Aspirate and wash four times with 250 µl/well of Wash buffer.
7. Dispense 100 µl of TMB substrate into each well.
8. Incubate 10 minutes (+/-5 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Keep the strips in the dark during the incubation with TMB substrate.
9. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate few times to ensure complete mixing of the reagents.
10. Measure the absorbance at 450 nm with a microplate reader within 10 minutes. It is recommended to use a reference reading at 630 nm.

Calculation

Begin the processing with subtraction of the absorbance of the DB well (background absorbance) from the absorbances in all other wells:

Processing of results for the Qualitative interpretation

1. Compute the absorbance mean of the three wells with Calibrator (Cal). If any of the three parallels absorbance is different from the mean in more than 20% then exclude the deviating well from the calculation and compute a new absorbance mean with using the other two wells.
2. Compute the cut-off value by multiplying the mean absorbance of Calibrator (Cal) with a Correction factor. The Correction factor for this Lot. (xxxxxx) is 0.xx.
3. Serum samples with absorbances lower than the cut-off value are considered negative and samples with absorbances higher than 130% of the cut-off value are considered positive. Serum samples with absorbance in the range 1.0-1.3 cut-off are equivocal.

Processing of results for the Semiquantitative interpretation

Determine Positivity Index for each serum sample as follows:

1. Compute the cut-off value
2. Compute the Positivity Index according to the following formula:

   sample absorbance = sample Positivity Index / cutoff value
3. Express the serum reactivity according to Table 1

Table 1: Semiquantitative interpretation of the results

Positivity index	Interpretation
< 1.0	Negative
1.0 - 1.30	+/-
1.31 - 2.00	+
2.01 - 3.00	++
> 3.00	+++

Reference Values

Validity of the test

The test is valid if:

1. The background absorbance (the absorbance of the Dilution buffer) is less than 0.1.
2. The Calibrator mean is higher than 0.7.
3. The ratio of mean absorbance of Positive control / cut-off is higher than 1.3.
4. The ratio of mean absorbance of Negative control / cut-off is lower than 0.9.

Precision

Intra assay variability: 3.1-4.2%

Inter assay variability: 2.3-13.1%

Sensitivity

Evaluation was performed by the comparing the VIDITEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

VZV status	Total	Positive	Equivocal	Negative	
Negative	180	3	10	167	**Specificity: 98.2%**
Positive	41	37	4	0	**Sensitivity: 100%**

Interferences

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides. Despite, examination of such a samples is not recommended. RF sorbent in Dilution buffer for the samples eliminate interference of rheumatoid factor in most samples. However, the sampls with very high level of RF may give false positive results, so as samples from patients with infectious mononucleosis or other conditions assaociated with polyclonal activation of antibody production.