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User's Manual

Cytomegalovirus (CMV) IgM ELISA

REF **DEIA466**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The ELISA kit is intended for the diagnosis of CMV-caused or CMV-associated diseases such as infectious mononucleosis, CMV syndrome or acute and chronical CMV infection in immunocompromised patients. The kit can also be used for complex characterization of chronic fatigue syndrome and for the determination of CMV seropositivity in blood and organs donors and recipients of these biological materials.

Principles of Testing

Cytomegalovirus (CMV) IgM ELISA is a solid-phase immunoanalytical test. The strips are coated with the mixture of recombinant antigens. Anti-CMV antibodies in serum samples bind to the mixture of immobilized antigens. Bound antibodies are later on recognized by animal anti-human IgM antibodies labelled with horseradish peroxidase. The amount of bound labeled antibodies is determined by color enzymatic reaction. Negative sera do not react and the mild change in color, if present, may be attributed to the reaction background.

Reagents And Materials Provided

1. ELISA 8-well break-away strips coated with a mixture of specific recombinant antigens 1x12pcs.
2. Negative control serum[1)], r.t.u., 1.3 mL,1 vial
3. Positive control serum, r.t.u., 1.3 mL, 1 vial
4. Calibrator, r.t.u, 1.3 mL, 1 vial
5. Anti-human IgM antibodies labelled with horseradish peroxidase r.t.u., 13mL, 1vial
6. Wash buffer 10x concentrated, 55 mL, 1vial
7. Dilution buffer r.t.u., 60 mL 1vial
8. Chromogenic substrate TMB, r.t.u., 13 mL, 1vial
9. Stop solution r.t.u. 13 mL, 1vial
10. RF sorbent[2)], 25x concentrated, 2 mL, 1vial
11. Instruction manual
12. Quality control certificate

[1)] (ready to use)

[2)] Goat anti-human IgG globulin

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Chromogenic substrate TMB) is compatible and interchangeable between ELISA-CD kits which contain [TMB and not compatible with other Chromogenic substrates used in other ELISA-CD TMB-O, TMB-BF

Materials Required But Not Supplied

Distilled or deionized water for dilution of the Wash buffer concentrate, appropriate equipment for pipetting,

liquid dispensing and washing, thermostat set at 37°C for ELISA plate incubation, spectrophotometer.

Storage

The ELISA kit should be used within three months after opening.

a. Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Under these conditions, the expiration period of the entire kit is indicated on the central label, the expiration date of the individual components is indicated on their package labels.

b. Store unused strips in the sealable pouch and keep the desiccant inside.

c. 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.

d. Store undiluted sera in aliquots at -18 to -28 °C. Frequent freezing and thawing is not recommended. Undiluted serum samples could be stored at +2 to +10°C up to one week.

e. Do not store diluted samples. Always prepare fresh.

Plate Preparation

	1	2	3	4	5	6	7	8	9	10	11	12
A	DIL PLUS	S4										
B	CAL	S···										
C	CAL											
D	PC											
E	NC											
F	S1											
G	S2											
H	S3											

Reagent Preparation

a. Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.

PREPARATION OF REAGENTS:

b. Vortex samples (sera), Calibrator and the Control sera in order to ensure homogeneity and mix all solutions well prior use. In the case of manual preformation of the test dilute the serum samples 101x in Dilution buffer Plus and mix (e.g. 5 uL of serum sample + 500 uL of Dilution buffer). Mix carefully and incubate 10 min. at room temperature. Dilution buffer Plus contains anti-human IgG antibodies for elimination of IgG antibodies and rheumatoid factor (RF). Diluted sera may form an opalescent solution. A precipitate does not interfere with the test performance. Do not dilute the Controls sera and Calibrator, they are ready to use.

c. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled/deionized water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of H_2O). 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 month if stored at room temperature.

d. Do not dilute anti-IgM/Px-conjugate, Chromogenic substrate TMB and stop solution, they are ready to use

(r.t.u.).

Assay Procedure

Manufacturer will not be held responsible for results if manual is not followed exactly.

Workflow for manual execution:

a. Allow the microwell strips sealed inside the aluminum 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 or seal it with vacuum.

b. Pipette 100 uL of Dilution buffer, Standards, Negative control and serum samples to the wells according to the pipetting scheme in Figure l: start with filling the first well dilution buffer (DIL PLUS), the next two wells with Calibrator CAL, next well with Positive control serum PC and another one well with Negative control serum NC and the remaining wells d. with tested sera (S1, S2, .). It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the samples in doublets and the calibrator CAJ in triplet. Incubate 30 minutes (+/-2 min) at 37°C.

c. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 ul/well of Wash buffer. Avoid cross-contamination 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.

d. Add 100 uL of anti-lg M Px-conjugate r.t.u CONJ-V into each well. Incubate 30 minutes (±2 min) at 37°C.

e. Aspirate and wash four times with 250 ul/well of Wash buffer (see point c).

f. Dispense 100 ul of T'MB substrate into each well. Incubate 15 minutes (± 30 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Follow this rule, avoiding a time difference. Pipette quickly in regular rhythm or use a suitable dispenser. Keep the strips in the dark during the incubation with TMB substrate.

g. Stop the reaction by adding 100 uL of Stop solution STOP. Use the same pipetting rhythm as with the TMB chromogenic substrate to ensure the same reaction time in all wells. Tap gently the microplate few times to ensure complete mixing of the reagents and without bubbles.

h. Measure the absorbance at 450 nm with a microplate reader within 10 minutes. It is recommended to use a reference reading at 620-690nm.

Calculation

Begin the processing of results with subtraction of the of the DIL PLUS well (background absorbance) from the absorbances in all other wells.

Processing of results for Qualitative interpretation

1. Compute the mean absorbance of the two wells with Positive control serum CAL. (If CAL) was applied in three parallels and in one the 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 of the test by multiplication the Calibrator with the correction factor. The correction factor value determined for this lot of the kit is stated in the Quality control certificate.

Serum samples with absorbances lower than the 90% of the cut-off value are considered negative and

samples with absorbances higher than 110% of the cut-off value are considered positive. Serum samples with absorbance in the range 90 % - 110 % cut-off are equivocal (grey-zone, see note) and then it is recommended to repeat testing or to test another sample from the patient, usually withdrawn 1-2 weeks later.

Processing of results for the Semiquantitative evaluation

Determine Positivity Index for each serum sample as follows:

1. Compute the cut-off value as in the previous evaluation.

2. Compute the Positivity Index according to the following formula:

Sample positivity index=sample absorbance/ cut-off value

3. Express the serum reactivity according to Semiquantitative interpretation of results.

Positivity index	Interpretation
<0.90	Negative
0.90-1.10	+/-
>1.10	Positive*

* On the basis of the Positivity Index value it is possible to estimate semi quantitatively the amount of antibodies in the sample

Example of calculation:

CAL absorbances =1.063;0.987;1.025

CAL mean = 1.025

Sample absorbance=0.800

Correction factor=0.32

Cut-off value = 1.025*0.32=0.328

Sample Positivity Index=0.800/0.328 = 2.44

Note! An equivocal sample reactivity. i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later,

Interpretation Of Results

Interpretation of the results must be done in context of patient's history, clinical symptoms and the results of other laboratory examinations. In immunocompromised patients CMV IgM negativity cannot exclude recent active infection. On the contrary, single CMV-IgM positivity does not provide proof of recent active infection. Samples from patients with acute EBV, HSV, VZV, Taxoplasma gondii, legionella or chlamydia infection or samples from patients with some autoimmune disorders may provide false positive results due cross-reactivity or polyclonal activation of antibody production. In pregnant women, supplementary serological tests, i.e. CMV IgG avidity should be taken into consideration.

Performance Characteristics

Validity of the test

The test is valid if:

The background absorbance (the absorbance of the DIL PLUS) is less than 0.150.

The mean absorbance values of standards/ control sera, and the ratio between the absorbance values of PC / CAL are in the ranges stated in the Quality control certificate for this kit lot.

Precision

Intra-assay precision:<8%

Inter-assay precision:<15%

Specificity

CMV

Interferences

Hemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of hemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides, but examination of such a sample is not recommended. RF sorbent in Dilution buffer for the samples eliminates interference of rheumatoid factor in most samples. However, the samples with very high level of RF may give false positive results.

Precautions

HANDLING PRECAUTIONS:

a. Manufacturer guarantees performance of the entire ELISA kit.

b. Wash solution, TMB substrate, Stop solution and Dilution buffer are compatible and interchangeable between ELISA-CD kits unless otherwise stated in the Instruction Manual. c. Work aseptically, avoid microbial contamination of serum samples and kit reagents.

d. During processing with reagents (removing, dilution and storage), avoid cross-contamination or contamination with enzyme-inhibiting substances.

e. Calibrator and control sera contain preservative ProClin 300@ (mix of 5-Chloro-2-methyl- 4-isothiazolin-3-one and 2-Methyl-2H-iothiazol-3-one (3: 1)).

f. Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.

g. Observe the instructions in the manual. Variations in the test results are usually due to: *Insufficient mixing of reagents and samples

*Inaccurate pipetting and inadequate incubation times

*Poor washing technique, spilling drops of the samples or Px-conjugate to the rim of wells *Use of the same pipette tip for different solutions or by swapping the caps

*Contamination of pipette which is used for sample, control and chromogen substrate TMB application with Px conjugate (we recommend for conjugate application use of a pipette reserved for this purpose only)

SAFETY PRECAUTIONS

All ingredients of the kit are intended for laboratory use only.

Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However, they should be regarded as contagious and handled and disposed of according to the appropriate regulations.

Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials for min 30 min with 3% chloramine.

Disinfect the wastes from the strip washing in a waste container using a suitable disinfectant solution (e.g. Incidur, Incidin, chloramine, ….) at a concentration recommended by the manufacturer. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly after wards. Avoid spilling or producing aerosol.

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