Version 23-04/24

User's Manual

Anti-VCA EBV IgA ELISA Kit

REF **DEIA336**

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

Anti-VCA EBV IgA ELISA Kit for the detection of IgA antibodies to Epstein Barr virus (EBV) capsid antigen (VCA) in human serum.

Principles of Testing

Anti-VCA EBV IgA assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with specific antigen that bear immunodominant epitopes of VCA complex. The anti-VCA 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 antihuman IgA 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.

Reagents And Materials Provided

ELISA break-away strips coated with specific antigen **STRIPSAg** 1 microplate

1. 1.3 mL Calibrator r.t.u.[1)] **CAL** 1 vial
2. 1.3 mL Positive control serum r.t.u. **PC** 1 vial
3. 1.3 mL Negative control serum r.t.u. **NC** 1 vial
4. 13 mL Anti-Human IgA antibodies labelled with horseradish peroxidase r.t.u (Px-conjugate) **CONJ** 1 vial
5. 55 mL Wash buffer 10x conc. **WASH 10x** 1 vial
6. 60 mL Dilution buffer r.t.u. **DIL** 1 vial
7. 13 mL TMB substrate r.t.u. **TMB** 1 vial
8. 13 mL Stop solution r.t.u. **STOP** 1 vial
9. Instruction manual
10. Certificate of quality

[1)] ready to use

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 kits which contain TMB and not with other Chromogenic substrates TMB-O, TMB-BF.

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. Thermostat (set at 37°) for ELISA plate incubation.
4. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

Storage

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

Store the kit and 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.

Store serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -18°C to -28°C. Avoid repeated thawing and freezing.

Do not store diluted serum samples. Always prepare fresh.

Kits are shipped in cooling bags, the transport time of 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.

Expiration date is indicated at the ELISA kit label and at all reagent labels.

Plate Preparation

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL**	**S4**										
b	**CAL**	**S…**										
c	**CAL**											
d	**PC**											
e	**NC**											
f	**S1**											
g	**S2**											
h	**S3**											

Reagent Preparation

1. Allow all kit components to reach room temperature.Turn on the thermostat to 37°C.
2. Vortex samples and the controls in order to ensure homogeneity and mix all solutions well prior use.
3. Dilute serum samples 1:100 in Dilution buffer and mix (5 µL of serum sample + 500 µL of Dilution buffer). Do not dilute the Control sera and Calibrator, they are ready to use.
4. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 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 month if stored at room temperature.
5. Mix well Px-conjugate and TMB substrate r.t.u.
6. Do not dilute TMB substrate and Stop solution and Px-conjugate they are ready to use.

Assay Procedure

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

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. Start with filling the first well with 100 µL of Dilution buffer **DIL** to estimate the reaction background. Fill next

two wells with 100 µL/well of Calibrator **CAL** and then fill next well with Positive control serum **PC** and another one well with Negative control serum **NC**.Fill the remaining wells with 100 µL of diluted serum samples (S1, S2, S3,...). It is sufficient to apply one serum into one well, however, if you wish to minimize laboratory error, apply the Calibrator **CAL** in triplet and samples and Controls in doublets. Incubate 30 minutes (±2 min) at 37 °C.

3. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Precautions). Wash and aspirate the wells four times with 250 µL/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.
4. Add 100 µL of Px-conjugate r.t.u. **CONJ** into each well. Incubate 30 minutes (±2 min) at 37 °C.
5. Aspirate and wash 4x with 250 µL/well of Wash buffer. Tap the plate on an adsorbent paper.
6. Dispense 100 µL of TMB substrate **TMB** into each well. Incubate for 15 minutes (+/- 30 seconds) in dark at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips and keep them in the dark during the incubation with TMB substrate.
7. Stop the reaction by adding 100 µL of Stop solution **STOP**. 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.
8. Measure the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 620-690 nm.

Quality Control

The test is valid if:

The background absorbance (the absorbance of the Dilution buffer) 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.

Calculation

Begin the processing of results with subtraction of the background absorbance (absorbance of the **DIL** well) from the absorbances of all other wells.If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

Processing of results for the qualitative interpretation

1. Compute the absorbance mean of the wells with Calibrator **CAL**. If the Calibrator was applied in three parallels and in one of them 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 by multiplying the mean of Calibrator with a correction factor. The correction factor value for Calibrator for this Lot is written in enclosed Quality control certificate.
3. Serum samples with absorbances lower than the 90% cut-off value are considered negative and samples with absorbances higher than the 110% of the cut-off value are considered positive.

Processing of results for the semiquantitative interpretation

Determine Positivity Index for each serum sample as follows:

1. Compute the cut-off value (see the previous paragraph)
2. Compute the Positivity Index according to the following formula:
   Sample absorbance Index = Sample Positivity / Cut-off value
3. Express the serum reactivity according to Semiquantitative interpretation of results:
   Semiquantitative interpretation of result:

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 semiquantitatively the amount of antibodies in the sample.

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.

Example of calculation:

Calibrator absorbances	= 0.986; 0.996; 0.998
Mean Calibrator absorbance	= 0.993
Correction factor	= 0.19
Cut-off value	= 0.993*0.19 = 0.189
Sample absorbance	= 0.800
Sample Positivity Index	= 0.800 / 0.189 = 4.23

Precision

The intraassay variability (within the test) and the interassay variability (between tests) were performed with samples of variable absorbance values.

Intraassay variability

The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot at least on 12 parallels of the same microtitration plate.

Example: (N = number of parallels of the same microtitration plate, ±σ = standard deviation)

N	Mean absorbance	±σ	CV%
16	1.335	0.050	3.8 %
16	0.614	0.023	3.7 %

Interassay variability

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the OD values of the same serum sample in several consecutive tests.

Example: (N = number of an independent examination of the same serum sample, ±σ= standard deviation):

N	Mean Absorbance	±σ	Range (min-max)	CV%
18	1.369	0.064	1.223-1.476	4.7 %
18	0.463	0.060	0.337-0.569	12.9 %
14	1.128	0.093	0.945-1.319	8.2 %

Recovery

Measured values of recovery test for every Lot are between 80-120% of expected values.

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.

Precautions

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 with 3% chloramine. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramin,...) in concentrations recommended by the producer. 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 afterwards. Avoid spilling or producing aerosol.

Handling Precautions

Manufacturer guarantees performance of the entire ELISA kit.

Follow the assay procedure indicated in the Instruction manual.

Wash buffer, TMB substrate, Stop solution and Dilution buffer are interchangeable between ELISA kits unless otherwise stated in the instruction manual.

Calibrator and control seracontain preservative ProClin 300®(mix of 5-Chloro-2-methyl-4- isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)).

Avoid microbial contamination of serum samples and kit reagents. Avoid cross-contamination of reagents.

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

Variations in the test results are usually due to:

Insufficient mixing of reagents and samples

Inaccurate pipetting and inadequate incubation times

Poor washing technique or spilling the rim of well with sample or Px-conjugate

Use of identical pipette tip for different solutions