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User's Manual

Human VCA EBV (CSF) IgG ELISA Kit

REF **DEIA470**

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

Human VCA EBV (CSF) IgG ELISA kit is intended for in vitro diagnostic procedures in EBV induced and EBV associated diseases, such as infectious mononucleosis (IM) and the chronic active EBV infection. The test is also usefull in the diagnosis of Burkitt's lymphoma, nasopharyngeal carcinoma, carcinoma of the Waldeyer's ring and in characterisation of opportunistic lymphomas (oligo- and polyclonal). The other use of the test is in characterisation of chronic fatigue syndrome, in neuroinfections and immunosupression which is frequently associated with EBV reactivation.

General Description

The Epstein–Barr virus (EBV), also called human herpesvirus 4 (HHV-4), is a virus of the herpes family, which includes herpes simplex virus 1 and 2, and is one of the most common viruses in humans. It is best known as the cause of infectious mononucleosis. It is also associated with particular forms of cancer, particularly Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and central nervous systemlymphomas associated with HIV. Finally, there is evidence that infection with the virus is associated with a higher risk of certain autoimmune diseases, especially dermatomyositis, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and multiple sclerosis. Most people become infected with EBV and gain adaptive immunity. In the United States, about half of all five-year-olds and 90–95% of adults have evidence of previous infection. Infants become susceptible to EBV as soon as maternal antibody protection disappears. Many children become infected with EBV, and these infections usually cause no symptoms or are indistinguishable from the other mild, brief illnesses of childhood. In the United States and in other developed countries, many people are not infected with EBV in their childhood years. When infection with EBV occurs during adolescence or teenage years, it causes infectious mononucleosis 35% to 69% of the time.

Principles of Testing

Human VCA EBV (CSF) IgG ELISA kit is an enzyme linked immunosorbent assay. The wells of polystyrene strips are coated with specific antigen containing immunodominant epitopes of the VCA complex. Anti-VCA antibodies if present in serum/CSF samples bind to the immobilized antigen. The antibodies that were bound to the antigen are in the next step of the assay detected with anti-human (IgG) antibodies labelled with horseradish peroxidase. The amount of the bound detection antibodies is measured by addition of a chromogenic substrate. A serum sample without antiVCAantibodies causes only a mild change of colour which, if occurs, may be attributed to the background of the reaction. Measurement of the intrathecal synthesis of anti-VCA antibodies reveals the antibody production within the central nervous system. Such measurements require quantification of the antibody response in paired serum and CSF samples, taken at the same time of the disease. The precise quantification is only possible if the sample reactivity is within the linear range of the ELISA calibration curve, therefore it is recommended to measure the presence of anti-VCA antibodies in two different serum dilutions. The calculation of the intrathecal synthesis requires knowledge of albumin and the total IgG concentration in both, the serum and the CSF sample. The calculation is done according to Reiber's equation.

Reagents And Materials Provided

1. 12x8 well **break away strips coated with antigen, framed**: 1 microplate
2. 1.3mL **Standard A**, 5 A.U./mL, ready to use: 1 vial
3. 1.3 mL **Standard B**, 16 A.U./mL, ready to use: 1 vial
4. 1.3 mL **Standard C**, 40 A.U./mL, ready to use: 1 vial
5. 1.3 mL **Standard D**, 95 A.U./mL, ready to use: 1 vial
6. 1.3 mL **Standard E**, 150 A.U./mL, ready to use: 1 vial
7. 1.3 mL **L Standard F**, 360 A.U./mL, ready to use: 1 vial
8. 0.2 mL **Anti-human IgG antibodies-Px conjugated (Pxconjugate)**, 101x concentrated: 1 vial
9. 125 mL **Wash buffer**, 10x concentrated: 1 vial
10. 125 mL **Dilution buffer (DB)**, ready to use: 1 vial
11. 15 mL **Chromogenic substrate (TMB substrate)**, ready to use: 1 vial
12. 30 mL **Stop solution**, ready to use: 1 vial
13. Zip lock plastic bag: 1 piece
14. Instruction manual: 1 piece
15. Certificate of quality: 1 piece

Materials Required But Not Supplied

1. Distilled / deionised water for diluting the concentrated Wash buffer.
2. Equipment for pipetting, dispensing and washing.
3. Test tubes for diluting samples, microplate cover or seal.
4. Microplate reader – wavelenght 450 nm.

Storage

1. Store the **unused strips** in the provided plastic bag with the desiccant kept inside.
2. Store **serum and CSF samples at temperature -18 to -28°C**, undiluted in small aliquots. Avoid repeated freezing and thawing of samples.
3. Short term storage (up to one week) of thawed/fresh serum samples is possible at +2 to +10°C.
4. Do not store the diluted samples and the diluted Pxconjugate, always prepare fresh.

Reagent Preparation

1. Allow all kit components to reach room temperature.
2. Vortex samples and Standards in order to ensure homogeneity prior use.
3. Dilute serum samples 1:100 times with Dilution buffer (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). In case of measurements of the intrathecal synthesis, prepare two serum dilutions (1:100 and 1:400), e.g. mix 50 µL of 1:100 diluted serum sample with 150 µL of Dilution buffer. Dilute cerebrospinal

fluid samples 1:1 in Dilution buffer (e.g. 100 µL of cerebrospinal fluid sample + 100 µL of Dilution buffer).

4. Do not dilute Standards, they are ready to use.
5. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with distilled/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 concentrate in a water bath set to 32-37°C. The 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 of Px-conjugate + 10 mL of Dilution buffer).
7. Do not dilute TMB substrate and Stop solution, they are ready to use.

Assay Procedure

Detection of serum antibodies only:

1. Allow the vacuum sealed strips to reach room temperature before opening. It prevents moist condensation within the wells. Withdraw the needed number of strips and put the remaining strips and the desiccant device in the provided plastic bag.
2. Pipette 100 µL of Dilution buffer (DB) to the first well to determine the reaction background. Continue with 100 µL of each Standard (A - F). Then pipette 100 µL of the diluted serum samples. If you want to minimize a laboratory error, pipette each sample in doublets. Incubate for 30 (+/- 2) minutes at laboratory temperature.
3. Aspirate the contents of the wells into a collecting bottle containing appropriate disinfectant. Wash and aspirate the wells four times with 250 µl/well of the diluted Wash buffer. Avoid well to well cross-contamination. Empty the wells by inverting the plate and tapping it against a pile of adsorbent papers.
4. Add 100 µL of the diluted Px-conjugate into each well.
5. Incubate for 60 (+/-5) minutes at room temperature.
6. Aspirate and wash as in step "c".
7. Dispense 100 µL of TMB substrate into each well. Incubate for 10 (+/-5 sec.) minutes at room temperature. The time measurement must be started at the beginning of TMB dispensing. Pipette in a regular rhythm or use a suitable dispensing instrument. Cover the strips with an aluminium foil or keep them in the dark during the incubation.
8. Stop the reaction by adding 100 µL of Stop solution. Use the same regular pipetting rhythm as mentioned above to ensure the same reaction time in all wells. Check the wells for bubbles and remove them by tapping gently the microplate for a few times.
9. Determine the absorbance at 450 nm in a microplate reader within 10 minutes. It is recommended to use a reference reading at 630 nm.

Calculation

Measurement of serum antibodies only:

1. Subtract the DB absorbance from the absorbances in all other wells (Blank difference data).
2. Construct the calibration curve by plotting the A.U./mL of Standards (x-axis) versus the absorbances of Standard wells (y-axis). It is recommended to use axis in logarithmic scale.

3. For each absorbance on the y-axis find the corresponding value on the x-axis. It is possible to use a variety of software applications for fitting the standard curve and calculation of the unknowns, e.g. Winliana, KimQ (4PL logistic) or MS Excell (linear regression – suitable only for the linear part of the calibration curve).
4. Multiply the results by the dilution factor (for sample dilution 1:100 – multiply the concentration in A.U./mL by 101).

Result interpretation

Concentration (A.U./mL)	Interpretation
< 1500	Negative
1500 - 2000	+/-
2001 - 4500	+
4501 - 11000	++
11001 - 24000	+++
>24000	++++

Interpretation Of Results

Result interpretation

VCA IgM	VCA IgG	VCA IgA	EA(D) IgG	EBNA-1 IgG	EBNA-1 IgM	EBV status
-	-	-	-	-	-	EBV negative
++	+-	++	+-	--	++	EBV primoinfection (acute phase)
++-	+++	++-	++-	+--	++-	EBV primoinfection (post-acute phase)
--	++	+-	--		--	EBV primoinfection (convalescent phase)
--	++	+-	--	++	--	Seropositivity without signs of the active EBV infection
+-	++	+-	++	++	--	EBV reactivation

Reference Values

Validity of the test

The test is valid if:

The absorbance of the DB well (reaction background) is less than 0.100.

The absorbance of the Standard D well is> 0.500.

The absorbances of Standards follow the order: ST A < ST B < ST C < ST D < ST E < ST F.

Dilution test

Samples having high, medium and low anti-VCA antibody titres were initially diluted 1:100 and then diluted further to get dilutions 1:200, 1:400 and 1:800. The resulting values were within the range of 100-113% of the expected value.

Precision

Intraassay variability: 4.9-5.2%

Interassay variability: 3.8-9.7%

Detection Limit

The limit of detection was calculated as the minimal concentration that was at the 95% confidence level different from the Blank. The limit of detection was 1,6 A.U./mL

Sensitivity

The diagnostic sensitivity was measured by testing the population sample that is expected to have anti-VCA IgG antibodies (blood donors, patients with active EBV infection). The results were confirmed by other commercial test. The diagnostic sensitivity was 98.1%.

Specificity

The specificity of the test was measured by testing EBV negative healthy blood donors. The specificity of the test was 97.1%.

Recovery

The analytical recovery was measured by testing samples repared as a mixture of a sample having optical density within the linear part of the calibration curve and of a sample having optical density maximally 10 times higher or 10 times lower. The resulting values were within the range of 97-114% of the expected value.

Interferences

Haemolytic, icteric and lipaemic samples showed no influence on results up to the concentration of 50 mg/mL of hemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides.