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User's Manual

Human Anti-Epstein Barr Virus (EBV) Nuclear Antigen 1 (EBNA-1) IgA ELISA Kit

REF **DEIA333**

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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

For the detection of IgA class antibodies against EBVEBNA-1 in human serum or plasma

General Description

The Epstein–Barr virus (EBV), also called human herpesvirus 4 (HHV-4), is a virus of the herpes family, and is one of the most common viruses in humans. It is the cause of infectious mononucleosis (glandular fever). It is also associated with particular forms of cancer, such as Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and conditions associated with human immunodeficiency virus (HIV) such as hairy leukoplakia and central nervous system lymphomas. 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. Infection with EBV occurs by the oral transfer of saliva and genital secretions.

Most people become infected with EBV and gain adaptive immunity. In the United States, about half of all five-year-old children and 90 to 95 percent of adults have evidence of previous infection. Infants become susceptible to EBV as soon as maternal antibody protection disappears. When infection with EBV occurs during adolescence, it causes infectious mononucleosis 35 to 50 percent of the time. The Epstein–Barr virus vaccine is not yet available. Gp350/220 and MVA-EL have been proposed as a target.

EBV can be divided into two major types, EBV type 1 and EBV type 2. These two subtypes have different EBNA-3 genes. As a result, the two subtypes differ in their transforming capabilities and reactivation ability. Type 1 is dominant throughout most of the world, but the two types are equally prevalent in Africa. EBV virus is approximately 120 nm to 180 nm in diameter and is composed of a double helix of DNA wrapped in a protein capsid. The capsid is surrounded by a tegument made of protein, which in turn is surrounded by a envelope made from lipids. The viral envelope contains glycoproteins, which are essential to infection of the host cell. Epstein–Barr nuclear antigen 1 (EBNA1) is a multifunctional, dimeric viral protein associated with Epstein–Barr virus (EBV). It is the only EBV protein found in all EBV-related malignancies. EBV infection may be identified by PCR and by the presence of antibodies (IgG, IgM and IgA) by ELISA. The optimal combination of serologic testing consists of the titration of four markers: IgM and IgG to the viral capsid antigen (VCA), IgM to the early antigen, and antibody to EBV nuclear antigen (EBNA). IgM to VCA appears early in infection and disappears within 4 to 12 weeks. IgG to VCA appears in the acute phase, peaks at 2 to 4 weeks after onset, declines slightly, and then persists for life. If antibodies to the viral capsid antigen are not detected, the patient is susceptible to EBV infection.

The optimal combination of serologic testing consists of the titration of four markers: IgM and IgG to the viral capsid antigen (VCA), IgM to the early antigen (EA), and antibody to EBVnuclear antigen-1 (EBNA-1). IgM to VCA appears early in infection and disappears within 4 to 12 weeks. IgG to VCA appears in the acute phase, peaks at 2 to 4 weeks after onset, declines slightly, and then persists for life. Anti-EA IgG appears in the acute phase of illness and generally falls to undetectable levels after 3 to 6 months. In many people, detection of antibody to EA is a sign of active infection. If antibodies to the viral capsid antigen are not detected, the patient is susceptible to EBV infection.

No approved EBV vaccine currently available. Several vaccines using EBV Gp350/220 and MVA-EL (modified vaccine Ankara-expressing EBV antigens: 280-aa from the C-terminus of EBNA1 and the full 497-

aa LMP2A fusion proteins) are in clinical trials.

Principles of Testing

The EBV EBNA-1 IgA antibody ELISA Kit is based on the principle of the enzyme immunoassay (EIA). EBV EBNA-1 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 IgA antibodies of the serum and the immobilized EBV EBNA-1 antibody takes place Diluted patient serum is added to wells coated with purified. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme that produced blue color. The intensity of the color generated is proportional to the amount of IgA specific antibody in the sample. 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 IgAantibodies is directly proportional to the intensity of the color.

Reagents And Materials Provided

EBV EBNA-1 antigens coated strip plate, (8x12 strip or 96 wells), 1 plate

Calibrator A (2 mL; Negative control), 1 vial

Calibrator B (2 mL; Cut-off standards), 1 vial

Calibrator C (2 mL; Weak positive control), 1 vial

Calibrator D (2 mL, Positive control), 1 vial

*Calibrator values are lot specific and specified on the vials.

Anti-Human IgA-HRP Conjugate, (15 ml), 1 bottle

Sample Diluent, 60 ml, 1 bottle

Wash buffer (10X) 60 ml, 1 bottle

TMB Substrate Solution, 15 ml, 1 bottle

Stop Solution, 15 ml, 1 bottle

Complete Instruction Manual, 1

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 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 (EDTA, 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 1:100 with ready-to-use sample diluent (e.g. 5 µL serum + 495 µL sample diluent). Do not dilute the calibrators.

Reagent Preparation

1. Dilute Wash buffer 1:10 with water. (Dilute 60 ml stock with 940 ml distilled water) Store diluted buffer at 4oC 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

Note: 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.

(ALLOW ALL REAGENTS TO REACH ROOMTEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute all samples 1:100 with the samplediluent. 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 (10X) 1:10 with distilled water.

1. Label or mark the microtiter well strips to be used on the plate
2. Dispense 100 ul diluent in 1 well to be used as blank. Pipet 100 ul of , calibrators, controls, and diluted samples (100:101) into appropriatewells in duplicate. See WORKSHEET OF A TYPICAL ASSAY. Cover the plate, mix gently for 5-seconds and incubate at room temp (25-28°C) for 60 min.

Wells	Stds/samples	Mean A450	Results
A1, A2	**Calibrator A Negative Control**		
B1, B2	**Calibrator B Cut-off standard**		
C1, C2	**Calibrator C Weak Positive**		
D1, D2	**Calibrator D Positive Control**		
E1, E2	Sample 1		Negative

NOTE: These data are for demonstration purpose only. Use the values that are generated with each test.

3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, wash the wells 3 times with 300 ul 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 ul anti-human IgA-HRP conjugate to all wells leaving oneempty for the substrate blank. Mix gently for 5-10 seconds. Cover theplate and incubate for 30 minutes at room temp (18-26°C).
5. Wash the wells 3 times as in step 3.
6. Add 100 ul TMB substrate solution. Mix gently for 5-10 seconds. Cover the plate and incubate for 20 minutes at room temp. Blue color develops in positive controls and samples.
7. Stop the reaction by adding 100 ul 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 60min.

Calculation

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

The OD of the calibrators (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.

The initial dilution of unknowns has been taken into consideration when reading the results from the graph. Results of unknowns of higher predilution have to be adjusted for the dilution factor.

Unknowns showing concentrations above the highest calibrator have to be diluted as described in "Test Procedure" and reassayed.

Values suggested by manual procedure:

IgA (U/mL)	Results
<8	negative
8-12	equivocal
>12	positive

In an in-house study apparently healthy research subjects showed the following results:

Ig isotype	n	Interpretations		
		positive	equivocal	negative
IgA	88	1.1 %	2.3 %	96.6 %

Precautions

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.

3. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
4. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
5. Before pipetting all reagents should be mixed thoroughly by gentle tiltingor swinging. Vigorous shaking with formation of foam should be avoided.
6. No reagents from different kit lots have to be used, they should not be mixed among one another.
7. All reagents have to be used within the expiry period.
8. In accordance with Good Laboratory Practices (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others
9. to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
10. The contact of certain reagents, above all the stopping solution and the substrate may cause skin or eye irritation. In the event of eye contact, rinse out immediately with plenty of water and consult a physician.