Human EBV EBNA1 IgM antibody ELISA Kit

Enzyme Immunoassay for the determination of EBV EBNA1 IgM in serum and plasma

Catalog number: ARG80546

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

In 1961 an infectious disease was identified in Uganda, which was correlated with the appearance of a defined type of tumor with children. The illness, which is found predominantly in Africa and Papua-New Guinea, was named Burkitt lymphoma from its discoverer. In 1964, Epstein, Barr and Achong characterized by electron microscopy as the causing agent a hitherto unknown virus, which belongs to the family of herpes viruses. The Epstein Barr virus is made responsible for a variety of diseases like infectious mononucleosis, Burkitt lymphoma, as well as nasopharyngeal carcinoma. In addition, a role of the virus is discussed in connection with Hodgkin´s disease. Especially with teenagers there appears a glandular fever syndrome, which is called „kissing disease“. Diseases which are caused by the Epstein Barr virus are found mainly in persons with reduced immunity. For example, the virus is associated with a lymphoproliferative disease which occurs after transplantation. The immune system of such patients is usually impaired by drug therapy. Also in immune-deficient AIDS patients, there appears frequently a state where cells at the rim of the tongue are infected (oral hairy leukoplakia). Infected persons keep the Epstein-Barr virus forever in their body, they are however mostly not ill. In the developing countries practically all the people are infected, in the western world the incidence is between 80% and 90%. The transmittance occurs already during childhood, perhaps by transfer from the mother, mainly via the saliva. During the active phase of the viral cycle, the Epstein-Barr virus produces about 100 different antigens, in the inactive phase around 10. The latter comprises among others the EBV nuclear antigen EBNA-1, which is closely correlated with a past infection and an immunity. The early antigen (EA) as well as the virus capsid antigen (VCA) from the active phase are also used as diagnostic markers. In a fresh infection, IgM antibodies against VCA and EA are

determined by immunofluorescence or ELISA. Later VCA IgG and afterwards EBNA-1 IgG antibodies appear. The simultaneous activation of VCA IgM and EBNA-1 IgG indicates correspondingly a reactivation of a latent EBV infection.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A specific EBV EBNA-1 antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any EBV EBNA-1 antibody present is bound by the immobilized antigen. After washing away any unbound substances, an HRP-conjugated antibody specific for human IgM is added to each well and incubate. Following the washing of any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of antigen-antibody binding in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of EBV EBNA-1 IgM in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Calibrator A (Negative Control)	2ml	4°C
Calibrator B (Cut-off Standard)	2ml	4°C
Calibrator C (Weak Positive Control)	2ml	4°C
Calibrator D (Positive Control)	2ml	4°C
HRP-conjugated antibody	15ml (Ready-to-use)	4°C
Sample Diluent	60ml	4°C
10X Wash buffer	60ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

For the performance of the test, the samples have to be diluted 1:101 with sample diluent.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Patient sample: Dilute patient sample 1:101 with Sample diluent buffer before assay, mix well. (e.g. 5 µl of serum + 500 µl of sample diluent buffer)

  Note: the controls are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of controls, diluted samples (1:101) and zero controls (sample diluent buffer) into wells. Cover plate and incubate for 1h at RT.
3. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl HRP-conjugated antibody (ready-to-use) into each well. Cover wells and incubate for 30 minutes at RT.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of TMB Reagent to each well. Incubate for 20 minutes at room temperature.

7. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

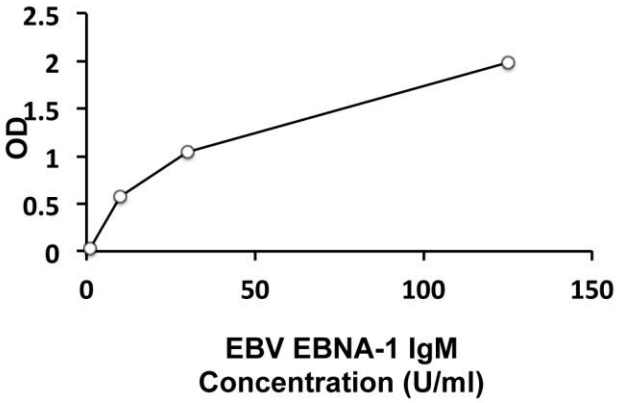
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



INTERPRETATION OF RESULTS

<8 U/ml (negative); 8-12 U/ml (Equivocal); >12 U/ml (Positive)

QUALITY ASSURANCE

Sensitivity

The mean MDD was 1.09 U/ml.

Assay Range: 1-150 U/ml

Specificity

No cross reactivity was observed with the following factors:

Cytomegalo, Herpes, Varicella

Intra-assay and Inter-assay precision

The CV value of intra-assay precision is 7.8% and inter-assay precision is 12.1%.

Inter-lot precision

4.5-12.9%

Recovery

82-118%

Linearity

76-119%