Human EBV EBNA1 IgG antibody ELISA Kit

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

Catalog number: ARG80545

Package: 96 wells

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 IgG 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.

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.
Calibrator A (Negative Control)	2ml	4°C
Calibrator B (Cut-off Control)	2ml	4°C
Calibrator C (Positive Control)	2ml	4°C
HRP-conjugated antibody	20ml (Ready-to-use)	4°C
Sample Diluent	100ml	4°C
20X Wash buffer	30ml	4°C
TMB substrate	14ml	4°C (Protect from light)
STOP solution	14ml	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 20X 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 20X 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 **37°C**.
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 **15 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. In order for an assay to be considered valid, the following criteria must be met:

   Substrate blank: Absorbance value **<0.100**

   Negative control: Absorbance value **<0.200 and <cut-off**

   Cut-off control: Absorbance value **0.350-0.850**

   Positive control: Absorbance value **>cut-off**

   If these criteria are not met, the test is not valid and must be repeated.

2. Calculation of Results

   The Cut-off is the mean absorbance value of the Cut-off Control determinations.

   Example: Absorbance value of Cut-off Control well 1 = 0.5

   absorbance value Cut-off control well 2 = 0.52

   Control mean absorbance = Cut-off = (0.5+0.52)/2 = 0.51

3. Results in Units [U]

   Units [U] = [Sample (mean) absorbance value x 10] / Cut-off

   Example: (1.2 x 10) / 0.51 = 23.5 U (Units)

   Note: Cut-off = (Cut-off X 10) / Cut-off = 10 U

4. Interpretation of results:

   - The samples are considered positive if the absorbance value is higher than 10% over the cut-off.

   - Samples with absorbance value of 10% above or below cut-off should be considered in the grey zone.

     It is recommended to repeat test again 2-4 weeks later with fresh sample. If the results in the second test are again in the grey zone, the sample has to be considered negative.

   - Samples are considered negative if the absorbance value is lower than 10% below the cut-off.

Summary:

	Unit	Note
Cut-off	10 U	-
Positive	> 11 U	Antibodies to Dengue Virus were detected.
Equivocal	9 – 11 U	Antibodies to Dengue Virus could not be detected clearly.
Negative	< 9 U	Antibodies to Dengue Virus could not be detected
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

QUALITY ASSURANCE

Intra-assay and Inter-assay precision

The CV value of intra-assay precision is 10.8% and inter-assay precision is 11.2%.

Recovery

77-93%

Linearity

96-187%