

Anti-EBNA 1 IgA ELISA Kit

Enzyme immunoassay for the detection of antibodies against EBNA1 IgA in serum and plasma

INTRODUCTION

The Epstein Barr Virus Nuclear Antigen-1 (EBNA-1) IgA Enzyme-linked Immunosorbent Assay (ELISA), is intended for the detection of IgA antibody to Epstein Barr Virus Nuclear Antigen-1 in human sera and plasma and help to diagnosis NPC.

SUMMARY AND EXPLANATION OF THE TEST

Detection of the Epstein-Barr virus was first described in 1964 by Epstein, Achong, and Barr using electron microscopic studies of cultured lymphoblasts derived from patients with Burkitt's lymphoma. EBV is classified as a member of the herpes-virus family based upon its characteristic morphology. EBV infection may demonstrate a wide spectrum of clinical symptoms. The majority of primary EBV infections are transmitted via saliva, occur during childhood, and are subclinical. In the U.S., 50% of the population demonstrate EBV antibodies before the age of 5 years; 80% by adulthood. Transfusion-associated EBV infections have also been reported. In young adults, EBV infection may be clinically manifested as Infectious Mononucleosis (IM) with typical symptoms of sore throat, fever, and lymphadenopathy. College students and military personnel are often cited as a high morbidity incidence population for IM. Infection of the target cells leads to two forms of viral cycles: 1) latent, nonproductive and 2) productive, replicative infections. In both cycles, one of the earliest antigens expressed is lymphocyte-detected membrane antigen, a cell surface antigen recognized by T-cells. It has been well established that most individuals exposed to EBV develop a heterophile antibody response. Expression of EBNA-1 either follows or parallels membrane antigen at 12 to 24 hours post infection. EBNA-1 is found as nonstructural, intranuclear antigen(s), present in all EBV-transformed cell lines as in tumors from Burkitt's and nasopharyngeal carcinoma patients. In the fully productive, replicative cycle, the synthesis of antigen follows EBNA-1. The viral capsid antigen complex (VCA) appears late in the replicative cycle. It has recently become apparent that EBNA-1 is probably not a single antigenic moiety, but a multicomponent antigen complex, on the basis of reactivities of sera from different classes of patients. The major component EBNA-1 has been purified and sequenced in its entirety.

Epstein-Barr virus has also been associated in the pathogenesis of two human cancers, Burkitt's lymphoma and nasopharyngeal carcinoma (NPC). Documentation by means of DNA hybridization studies demonstrates the presence of the EBV genome on biopsy specimens taken from individuals with these carcinomas. Burkitt's lymphoma is primarily observed in Sub-Saharan Africa, especially in African children, and in New Guinea. Malarial infections are usually diagnosed in Burkitt's lymphoma patients and are suggested to be a co-factor. Nasopharyngeal carcinoma is observed in Asia, most notably in Southern China, and may have genetic or environmental influences as the co-factor.

Serological studies have shown that the clinical onset of NPC is preceded by the appearance of a high antibody titer of IgA to viral capsid antigens and early antigens. The titers increase with the total tumor burden and the antibodies decline with the response to therapy. In patients with confirmed clinical remission elevation of IgA serological titers is highly significant for prediction of relapse

PRINCIPLE OF THE TEST

Purified EBNA-1 antigen is coated on the surface of microwells. Diluted patient serum is added to wells, the anti-EBNA specific antibody, if present, will bind to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kits:

1. HCV Plate: 8x12 or 12x8 wells coated with recombinant EBNA1 antigen.
2. Specimen Diluent: 12 ml chemically defined solution containing proteins, Tween 20, and sodium azide in phosphate buffer
3. Positive Control: 0.50ml
4. Negative Control: 0.50ml
5. Enzyme Conjugate: 12 ml Goat anti-human-IgA HRP Conjugate.
6. Substrate Solution A: 6 ml HRP Substrate.
7. Substrate Solution B: 6 ml TMB.

8. Concentration Washing Solution (20X): 40ml phosphate buffered saline solution with Tween 20. The buffer should be diluted 20 times with distilled water before use.
9. Stop Solution: 6 ml 2N sulfuric Acid.

Materials required but not provided:

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

PRECAUTION FOR USERS

All human source material used in the preparation of this product was found to be negative for the presence of HIV-1/HIV-2 antibodies, as well as for the hepatitis B surface antigen, using a commercial licensed method. Nevertheless, because no test method can offer complete assurance of the absence of infectious agents, this product should be handled with caution.

1. Avoid contact of reagents with the eyes and skin. If that occurs, wash thoroughly with water.
2. Wear gloves.
3. Do not pipette by mouth.
4. Do not smoke.
5. Dispose all used materials in a suitable biohazardous waste container. Remains of samples, controls, aspirated reagents and pipette tips should be collected in a container for this purpose and autoclaved 1-hour at 121°C or treated with 10% sodium hypochlorite (final concentration) for 30 min before disposal. (Remains containing acid must be neutralised prior addition of sodium hypochlorite).
6. Adjust washer to the plate used (flat bottom) in order to wash properly.
7. Do not mix reagents from different lots.
8. Do not use reagents after expiration date.
9. Extreme care should be taken to avoid microbial contamination and cross contamination of reagents.
10. Use a new pipette tip for each specimen and each reagent.
11. Soaps and/or oxidising agents remaining in containers used for the substrate-TMB solution can interfere with the reaction.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURE

1. Dispense 100µl of specimen diluent into individual test wells.
2. Dispense 100µl positive control and negative control duplicate into individual wells.
3. Add 10µl of each test sample into duplicate test wells; vortex to mix.
4. Incubate for 30 minutes at 37°C
5. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
6. Add 100µl of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minutes. Do not add Enzyme Conjugate to the blank well.
7. Incubate for 20 minutes at 37°C
8. Wash the plate 5 times as step 6.
9. Add one drop (50µl) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50µl) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 15 minutes. .
10. Add one drop (50µl) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader.

RESULT INTERPRETATION

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, The positive control OD value should be ≥ 0.6 , the negative control should be ≤ 0.10):

Cut-off Calculations:

Take average OD values of Negative control and add 0.10:

$$1x \text{ NC} + 0.10 = \text{Cut-off.}$$

Positive OD reading: \geq Cut-off value

Negative OD reading: $<$ Cut-off value

LIMITATIONS OF THE ASSAY

1. As the other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of entire content of wells completely before adding the washing solution.
2. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.
3. Samples with positive or equivocal result must be reanalysed in duplicate. If both retest values are lower than the cut-off, the final interpretation of the test is negative for EBV antibodies. If the result is repeatedly positive or equivocal, the sample should be further investigated with other methods.
4. Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.

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Biocare Diagnostics Ltd.

6F, Building B, 108 Xinghua Road

Xiangzhou, Zhuhai, China 519000

Tel: +86-756-8238560

E-mail: info@ivdbiocare.com

Website: www.ivdbiocare.com

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In vitro diagnostic device



Consult instructions for use



Keep dry



Temperature limitation



Use by



Lot code



Catalogue number



Contains sufficient for <n> tests



Manufacturer



Do not use if package damaged