Epstein Barr Virus Zta IgA ELISA Kit

Cat. No.:DEIA1034
Pkg.Size:96T

Intended use

For the qualitative determination of IgA class antibodies against Epstein Barr Virus in Human serum or plasma. It is intended for diagnosing and monitoring of patients related to infection by Epstein Barr Virus.

General Description

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 it's 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.

Principle Of The Test

This kit employs solid phase, indirect ELISA assay for detection of IgA antibodies to EBV Zta in two-step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant EBV Zta. During the first incubation step, EBV Zta IgA specific antibodies, if present, will be bound to the solid phase pre-coated antigen complexes. The wells are washed to remove unbound serum proteins, and anti-human IgA antibodies (anti-IgA) conjugated to horseradish peroxidase (HRP) are added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-IgA complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-(IgA)-anti-IgA (HRP) immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for IgA antibodies to EBV Zta remain colorless.

Reagents And Materials Provided

Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with Purified EB-Zta antigen
Positive control, human serum: 1 ml
Negative control, human serum: 1 ml
HRP-conjugated anti-human IgA antibodies: 11 ml
Sample Diluent: 11 ml
Wash Buffer (25×): 30 ml
Chromogen Solution A, Urea peroxide solution: 6 ml
Chromogen Solution B, TMB solution-Tetramethylbenzidine dissolved in citric acid: 6 ml
Stop Solution, 2M sulfuric acid: 6 ml

Materials Required But Not Supplied

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1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multi-channel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37 °C incubator.

Storage

Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles.

Specimen Collection And Handling

Use Human serum or plasma samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Reagent Preparation

1. Coated snap-off Strips: The ready to use break apart snap-off strips are coated with Purified EB-Zta antigen. Store at 2-8 °C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8 °C; stability until expiry date.
2. Washing Solution (25x conc.): The bottle contains 30 ml of a concentrated buffer, detergents and preservatives. Dilute Washing Solution 1+24; e.g. 10 ml Washing Solution + 240 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. After first opening stability until expiry date when stored at 2-8 °C.

Assay Steps

1. Bring all reagents to room temperature (18 - 25°C) before use. It is recommended that all samples or controls should be run at least in duplicate.
2. Add 100µl Sample Diluent into each well except in the blank.
3. Add 5µl of Specimen and 100µl of controls into their respective wells.
4. Seal the plate with the cover and incubate at 37°C for 60 min.
5. Discard the solution and wash 5 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µl of HRP-conjugated anti-human IgA antibodies to each well and incubate at 37°C for 60 min.
7. Wash microplate as described above.
8. Add 50µl of Chromogen A and 50µl Chromogen B solutions into each well including the Blank. Mix gently, protected from light and incubates at 37°C for 15-20 min.
9. Stop the reaction by adding 50 µl of Stop Solution to each well.
10. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. Read optical density at 450 nm with a microtiter reader within 15 minutes.

Quality Control

In order for an assay to be considered valid, the following criteria must be met:
• Substrate blank: Absorbance value < 0.08.
• Negative control: Absorbance value < 0.12.
• Positive control: Absorbance value 0.8 – 2.4.

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

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<10%

Limitations

1. Non-repeatable positive result may occur due to the general biological characteristics of the ELISA method. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the “indirect” model minimizes the unspecific reactions due to interference with unknown matters in sample and the anti-human IgA antibodies. Antibodies may be undetectable during the early stages of the
disease and in some immunosuppressed individuals.
2. Positive results must be confirmed with another available method and must be interpreted together with the patient clinical information and other laboratory results like X-ray and microbiolog.
3. Common sources for mistakes are: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. The prevalence of the marker will affect the assay’s predictive values.
5. If, after retesting of the initially reactive samples, the assay results are negative , these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results cannot be use to measure antibodies concentrations.

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