OriGene Technologies, Inc.
9620 Medical Center Dr., Suite 200, Rockville, MD 20850
Phone: 1.888.267.4436 Fax: 301-340-9254
Email: techsupport@origene.com Web: www.origene.com

Product Information

EBV-VCA IgA ELISA kit

Catalog Number: EA100940
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The EBV-VCA IgA ELISA tests system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgA class antibodies to EBV in human serum or plasma.

Background

Epstein-Barr virus (EBV) is a herpes virus known to cause infectious mononucleosis (IM). EBV infection may demonstrate a wide spectrum of clinical symptoms. The majorities of primary EBV infections are transmitted via saliva, occur during childhood, and are sub-clinical. 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. Epstein-Barr virus has also been associated in the pathogenisis of two human cancers, Burkitt's lymphoma and nasopharyngeal carcinoma. Burkitt's lymphoma is primarily observed in Sub-Sahara Africa, especially in African children, and in New Guinea. Nasopharyngeal carcinoma is observed in Asia, most notably in Southern China.

Principle of the Test

Diluted patient serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen IgA specific antibody, if present, binds to the antigen. 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 oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgA specific antibody in the sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with EBV-VCA antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: 1 Vial (ready to use)	1ml
4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Place the desired number of coated strips into the holder.

2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl l of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of a Standard Curve

Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

Quality Control

The test run may be considered valid provided the following criteria are met:

1. If the O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be greater than 1.2.

Interpretation

The following is intended as a guide to interpretation of EBV-VCA IgA test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

- **Antibody Index Interpretation**
  - <0.9 No detectable antibody to EBV-VCA IgA by ELISA
  - 0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
  - >1.1 Detectable antibody to EBV-VCA IgA by ELISA

Limitations of the Test

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. Lipemic or hemolyzed samples may cause erroneous results.

Performance Characteristics

Sensitivity and Specificity

98 sera from patients with suspected EBV infection were tested by this EBV-VCA IgA ELISA and a reference ELISA method. 14 sera were positive and 79 were negative by both methods (95% agreement). The results are summarized below:

		EBV-VCA IgA ELISA (EA100940)		Total
		+	-	
Reference ELISA kit	+	14	2	16
	-	3	79	82
Total		17	81	98

References

1. Gray JJ. Avidity of EBV VCA-specific IgG antibodies: distinction between recent primary infection, past infection and reactivation. J Virol Methods 1995;52(1-2):95-104.
2. Liu MT; Yeh CY. Prognostic value of anti-Epstein-Barr virus antibodies in nasopharyngeal carcinoma (NPC). Radiat Med 1998;16(2):113-7.
3. Hadar T; Margalith M; Sagiv E; Sarov B; Sarov I. The significance of serum IgM IgA and IgG antibodies specific for Epstein-Barr virus as determined by immunoperoxidase assay in the rapid diagnosis of infectious mononucleosis. Isr J Med Sci 1995;31(5):280-3.
4. Levine PH; Stemmermann G; Lennette ET; Hildesheim A; Shibata D; Nomura A. Elevated antibody titers to Epstein-Barr virus prior to the diagnosis of Epstein-Barr-virus-associated gastric adenocarcinoma. Int J Cancer 1995;60(5):642-4.
5. Debyser Z; Reynders M; Goubau P; Desmyter J. Comparative evaluation of three ELISA techniques and an indirect immunofluorescence assay for the serological diagnosis of Epstein-Barr virus infection. Clin Diagn Virol 1997;8(1):71-81.

Version 3, last updated October 18, 2015