9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

EBV-VCA IgM ELISA Kit

Name	EBV-VCA IgM ELISA Kit
INTENDED USE	The EBV-VCA IgM ELISA tests system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgM class antibodies to EBV in human serum or plasma.
SUMMARY AND EXPLANATION	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. IgM 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 IgM specific antibody in the sample.

MATERIALS PROVIDED	96 Tests
Microwells coated with EBV-	12x8x1

VCA antigen	
Sample Diluent: 1 bottle (ready to use)	22 ml
Calibrator: 1 Vial (ready to use)	1ml
Positive Control: 1 vial (ready to use)	1ml
Negative Control: 1 vial (ready to use)	1ml
Enzyme conjugate: 1 bottle (ready to use)	12ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8 C.

2. Keep microwells sealed in a dry bag with desiccants.

3. The reagents are stable until expiration of the kit.

4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease

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	Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
SPECIMEN COLLECTION AND HANDLING	1. Collect blood specimens and separate the serum. 2. Specimens may be refrigerated at 2–8 C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.
REAGENT PREPARATION	Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C).
ASSAY PROCEDURE	Bring all specimens and kit reagents to room temperature (20-25C) and gently mix. Place the desired number of coated strips into the holder. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 l of the sample to 200 l of sample diluent. Mix well. Dispense 100 l of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100l 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. Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 l of enzyme conjugate to each well and

	incubate for 20 minutes at room temperature. Remove enzyme conjugate from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 l of TMB substrate and incubate for 10 minutes at room temperature. Add 100 L of stop solution. 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	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. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF). Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value. Example of typical results: 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
LIMITATIONS OF THE TEST	To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and shows no interference with test results. It can be removed by centrifugation. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely. 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.

Lipemic or hemolyzed samples may cause erroneous results.

QUALITY CONTROL

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

The O.D. of the Calibrator should be greater than 0.250.

The Ab index for Negative control should be less than 0.9.

3. The Ab Index for Positive control should fall within the range

specified on the COA/label.

INTERPRETATION

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

Antibody Index Interpretation

1.1 Detectable antibody to EBV-VCA IgM by ELISA.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

92 patient sera were tested by this EBV-VCA IgM ELISA and a reference ELISA method. 18 sera were positive and 72 were negative by both methods (98% agreement). The results are summarized below:

	EBV-VCA IgM ELISA	EBV-VCA IgM ELISA	EBV-VCA IgM ELISA
	+		Total
Reference ELISA Kit +	18	1	19
_	1	72	73
Total	19	73	92

Precision

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %

1	16	1.48	0.055	3.80
2	16	1.93	0.027	2.90
3	16	0.29	0.015	5.26

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.85	0.143	7.75
2	10	0.86	0.075	8.72
3	10	0.5	0.047	9.40

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