ab178660 – Anti-Epstein Barr virus (EBV-VCA) IgA ELISA Kit

Instructions for Use

For the qualitative measurement of IgM class antibodies against Epstein Barr Virus in Human serum and plasma (citrate).

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

Version 1 Last Updated 11 November 2013

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1. BACKGROUND

Abcam's anti-Epstein Barr virus (EBV-VCA) IgA i*n vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate qualitative measurement of IgA class antibodies against Epstein Barr virus in Human serum and plasma.

A 96-well plate has been precoated with Epstein Barr Virus antigens to bind cognate antibodies. Controls or test samples are added to the wells and incubated. Following washing, a horseradish peroxidase (HRP) labelled anti-Human IgA conjugate is added to the wells, which binds to the immobilized with Epstein Barr Virus antigens. TMB is then catalyzed by the HRP to produce a blue color product that changes to yellow after adding an acidic stop solution. The density of yellow coloration is directly proportional to the amount of with Epstein Barr Virus antigens IgA sample captured in plate.

Epstein Barr Virus (EBV) is a member of the herpesvirus family (Gamma subgroup, DNA virus of 120-200 nm) and one of the most common human viruses. The virus occurs worldwide, and most people become infected with EBV sometime during their lives. Transmission of the virus is almost impossible to prevent since many healthy people can carry and spread the virus intermittently for life. Infants become susceptible to EBV as soon as maternal antibody protection disappears. Infection of children usually causes no symptoms. Infection during adolescence or young adulthood causes infectious mononucleosis 35% to 50% of the time.

Infectious mononucleosis is almost never fatal. There are no known associations between active EBV infection and problems during pregnancy, such as miscarriages or birth defects. Although the symptoms of infectious mononucleosis usually resolve in 1 or 2 months, EBV remains dormant or latent in a few cells in the throat and blood for the rest of the person's life. Periodically, the virus can reactivate and is commonly found in the saliva of infected persons. This reactivation usually occurs without symptoms of illness.

EBV also establishes a lifelong dormant infection in some cells of the body's immune system. A late event in a very few carriers of this virus

is the emergence of Burkitt´s lymphoma and nasopharyngeal carcinoma, but EBV is probably not the sole cause of these malignancies.

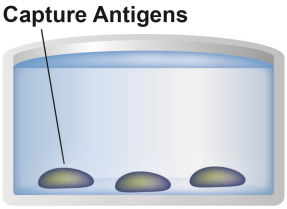
Species	Disease	Symptoms	Mechanism of infection
Epstein-Barr Virus	Infectious mononucleosis	Fever, sore throat, swollen lymph glands	Person to person transmission. EBV requires intimate contact with the saliva of an infected person, but the virus is also found in the saliva of healthy people

The presence of bacterial respiratory infection may be identified by

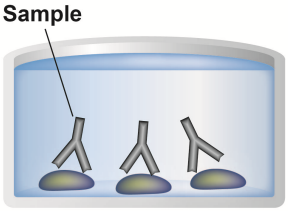
- PCR
- Serology: “mono spot” test, Detection of antibodies by ELISA

The optimal combination of serologic testing consists of the titration of five markers: IgA, IgG and IgM to the viral capsid antigen (VCA), antibody to the early antigen, and antibody to EBV nuclear antigen (EBNA). IgA and IgM to VCA appear early in infection and disappear within 4 to 12 weeks. IgG to VCA appears in the acute phase, peaks at 2 to 4 weeks after onset, declines slightly, and then persists for life. If antibodies to the viral capsid antigen are not detected, the patient is susceptible to EBV infection.

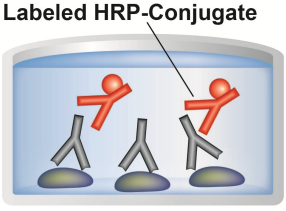
2. ASSAY SUMMARY



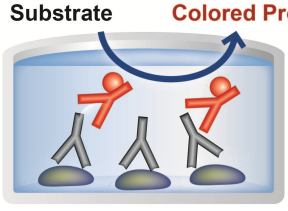
Prepare all reagents, samples and controls as instructed.



Add samples and controls to wells used. Incubate at 37°C.



Wash each well and add prepared labeled HRP-Conjugate. Incubate at room temperature.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Epstein Barr Virus (IgA) Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
IgA Sample Diluent***	100 mL	2-8°C
Stop Solution	15 mL	2-8°C
20X Washing Solution*	50 mL	2-8°C
Epstein Barr Virus anti-IgA HRP Conjugate**	20 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Epstein Barr Virus IgA Positive Control***	2 mL	2-8°C
Epstein Barr Virus IgA Cut-off Control***	3 mL	2-8°C
Epstein Barr Virus IgA Negative Control***	2 mL	2-8°C
Strip Holder	1 unit	2-8°C
Cover Foil	1 unit	2-8°C

* Contains 0.1 % Bronidox L after dilution

** Contains 0.2 % Bronidox L

*** Contains 0.1 % Kathon

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi and single channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 20X Washing Solution with deionized water. To make 200 mL 1X Washing Solution combine 10 mL 20X Washing Solution with 190 mL deionized water. Mix thoroughly and gently.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- Use Human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days of sample collection, the specimen should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -80°C). If samples are stored frozen, mix thawed samples well before testing.

  Avoid repeated freezing and thawing.

11. SAMPLE PREPARATION

- Before assaying, all samples should be diluted 1:100 with IgA Sample Diluent. Add 10 µL sample to 990 µL IgA Sample Diluent to obtain a 1:100 dilution. Mix gently and thoroughly.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Reliability of results depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **All controls (Epstein Barr Virus IgA Positive, Epstein Barr Virus IgA Negative and Epstein Barr Virus IgA Cut-off) must be included with each assay performed to determine test results.**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 100 µL of controls or diluted sample into appropriate wells. Leave one well for substrate blank.

13.4. Cover wells with the foil supplied in the kit and incubate for 1 hour at 37°C.

13.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

Note: Complete removal of liquid at each step is essential for good assay performance.

13.6. Add 100 µL Epstein Barr Virus anti-IgA HRP Conjugate into all wells except for the blank well. Cover with foil.

13.7. Incubate for 30 minutes at room temperature. Do not expose to direct sunlight.

13.8. Repeat step 13.5.

13.9. Add 100 µL TMB Substrate Solution into all wells

13.10. Incubate for exactly 15 minutes at room temperature in the dark.

13.11. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.

Note: Any blue color developed during the incubation turns into yellow.

13.12. Highly positive samples can cause dark precipitates of the chromogen. These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution for example 1:1 is recommended. Then dilute the sample 1:100 with IgA Sample Diluent and multiply the results in Standard Units by 2 (See Section 14. Calculations.)

13.13. Measure the absorbance of the specimen at 450 nm within 30 minutes of addition of the Stop Solution.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

14. CALCULATIONS

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 **0.150 – 1.300**
- **Positive control:** Absorbance value **> cut-off**

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

Calculation of Results

Calculate the mean background subtracted absorbances for each sample and compare to mean Cut-off control value.

The Cut-off control value is the mean absorbance value of the Cut-off control wells.

Example: Absorbance value Cut-off control Well 1 = 0.39

Absorbance value Cut-off control Well 2 = 0.37

Mean Cut Off value: (0.39 + 0.37)/2 = 0.38

Interpretation of Results

Samples are considered to give a positive signal if the absorbance value is greater than 10% over the cut-off value.

Samples with an absorbance value of less than 10% above or below the Cut-off control value should be considered as inconclusive (grey zone) i.e. neither positive or negative. It is recommended to repeat the assay using fresh samples. If results of the second test are again less than 10% above or below the Cut-off control value the sample has to be considered negative.

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

Results in Standard Units

$$\frac{\text{Patient (mean) absorbance value x 10}}{\text{Cut-off}} = \text{Standard Units}$$

Example: $\frac{1.786 \times 10}{0.38} = 47$ *Standard Units*

Cut-off:	10	Standard Units
Grey zone:	9-11	Standard Units
Negative:	<9	Standard Units
Positive:	>11	Standard Units

15. TYPICAL SAMPLE VALUES

PRECISION –

Positive Serum	**Intra-Assay**	**Inter-Assay**
n=	8	3
Mean	1.228	1.273
%CV	4.8	8.4

16. ASSAY ANALYTICAL SPECS

SPECIFICITY -

The specificity is > 95 % and is defined as the probability of the assay scoring negative in the absence of the specific analyte.

SENSITIVITY -

The sensitivity is > 95 % and is defined as the probability of the assay scoring positive in the presence of the specific analyte.

17. INTERFERENCES

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.2 mg/mL bilirubin.

18. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

19. NOTES

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