Epstein-Barr Virus (VCA) IgA ELISA Kit (Human) (OKNX00133)

Instructions for use

For the qualitative detection of Anti-Epstein-Barr Virus IgA in human serum.

This product is intended for research use only.

Lot to lot kit variations can occur. Use the kit manual which has been provided along with the kit packaging.

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1. Background

Principle

Aviva Systems Biology Epstein-Barr Virus (VCA) IgA ELISA Kit (Human) (OKNX00133) is based on standard reverse capture sandwich enzyme-linked immuno-sorbent assay technology. Epstein-Barr Virus antigen has been pre-coated and blocked in a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and washed. An HRP conjugated detector antibody specific for Human IgA is added, incubated and followed by washing. An enzymatic reaction is produced through the addition of substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration is read by absorbance at 450 nm and is qualitatively proportional to the amount of sample anti-Epstein-Barr Virus IgA captured the in well.

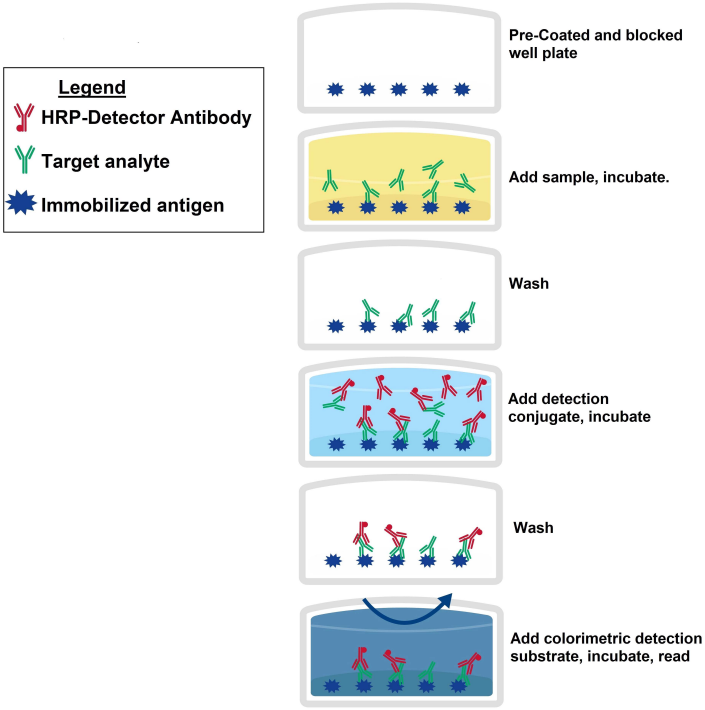
Background

Epstein-Barr Virus (EBV) is a member of the herpes virus 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 (VCA)	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 pathogen or infection may be identified by:
PCR
Serology: "mono spot" test, Detection of antibodies by ELISA

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at 4°C for 6 months.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description		Quantity	Storage Conditions
Epstein-Barr Virus IgA Microplate		96 Wells (12 x 8 Well strips)	4°C for 6 Months
Sample Diluent	White Cap	1 x 100 mL	
Stop Solution	Red Cap	1 x 15 mL	
20X Wash Solution	White Cap	1 x 50 mL	
Anti-Human IgA HRP Conjugate	Black Cap	1 x 20 mL	
TMB Substrate	Yellow Cap	1 x 15 mL	
Epstein-Barr Virus IgA Cut-Off Control	Green Cap	1 x 3 mL	
Epstein-Barr Virus IgA Positive Control	Red Cap	1 x 2 mL	
Epstein-Barr Virus IgA Negative Control	Blue Cap	1 x 2 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

• The following reagents are provided at ready to use concentrations and require no preparation:

- Sample Diluent
- Stop Solution
- Anti-Human IgA HRP Conjugate
- TMB Substrate
- Epstein-Barr Virus IgA Cut-Off Control
- Epstein-Barr Virus IgA Positive Control
- Epstein-Barr Virus IgA Negative Control

8.1 1X Wash Buffer

8.1.1 If crystals have formed in the 20X **Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.1.2 For 500 mL, add 25 mL contents of the 20X **Wash Buffer** bottle to 480 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.1.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.1.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.2 Microplate Preparation

• Micro-plates are provided ready to use and do not require rinsing or blocking.

• Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

• Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C.Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• Serum - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• Plasma - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

9.2 Sample Dilution (1:100)

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

• Dilute samples using **Sample Diluent**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

The suggested 100-fold dilution can be achieved by adding 10 µL sample to 990 µL of **Sample Diluent**.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for inter- and intra-assay reproducibility will be observed when incubation at 37°C as indicated in the procedure below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch. Allow additional wells to include the following (recommended in duplicate):

10.1.1 Substrate Blank

10.1.2 Negative Control

10.1.3 Cut-Off Control

10.1.4 Positive Control

10.2 Add 100 µL of the **1:100 diluted samples**, **Positive Control, Cut-Off Control** or **Negative Control** to test wells of the Epstein-Barr Virus IgA Microplate. At least two replicates are recommended.

10.3 Cover the plate with the well plate lid and incubate at 37±1°C for 60±5 minutes.

10.4 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Wash plate 3 times with **1X Wash Buffer** as follows:

10.6.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.6.2 Allow to soak for ~ 5 seconds.

10.6.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.6.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6.5 Repeat steps 10.6.1 through 10.6.4 **two** more times.

10.7 Add 100 µL of the **1X Anti-Human IgA HRP-Conjugate** to all the wells.

10.8 Cover with the well-plate lid and incubate at room temperature for 30 minutes.

10.9 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.10 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.11 Repeat the wash in step 10.6.

10.13 Add 100 µL of **TMB Substrate** to each well and incubate at room temperature **in the dark** for 15 minutes.

10.14 Add 100 µL of **Stop Solution** to each well in the same order as that of step 10.13.

10.15 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.14. If wavelength correction is available, set to 620 nm.

11. Calculation of Results

A positive or negative Epstein-Barr Virus IgA determination is derived by comparing the test samples to the **Positive** and **Negative Controls**.

11.1 Calculation of Results

- The **Cut-Off** is the mean 450 nm absorbance (A_{450}) value of the **Cut-Off Control** sample determinations.
- The Test Specimen measurement is the mean of the replicate A_{450} measurements.

11.2 Run Validation Criteria

For an assay to be considered valid, the following criteria must be met:

Substrate blank: Absorbance value < 0.100.

Epstein-Barr Virus IgA Negative control: Absorbance value < 0.200 and < Cut-Off

Epstein-Barr Virus IgA Cut-off Control: Absorbance value 0.150 – 1.30

Epstein-Barr Virus IgA Positive control: Absorbance value > Cut-Off

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

11.3 Interpretation of Results

Cut-off	10 NTU	
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine)
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

12 Typical Expected Data

12.1 Precision

Replicate samples were measured to assess inter- and intra-assay reproducibility.

Metric	Sample Type	n=	Mean	CV%
Inter-Assay	17016	12	12.4	4.5
Inter-Assay	A3	12	21.4	3.6
Intra-Assay	17016	20	0.548	5.5
Intra-Assay	A3	23	0.914	3.6

12.2 Specificity

Specificity is determined as the probability of the assay indicating a negative score in samples absent of the specific analyte:100%

12.3 Sensitivity

Sensitivity is determined as the probability of the assay indicating a positive score in samples with the specific analyte present: 100%

12.4 Interference Factors

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.5 mg/ml bilirubin.

12.5 Cross-Reactivity

Investigation of a specimen panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of
false-positive results due to cross-reactions.

13 Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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