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ab222940 Anti-Epstein-Barr Virus IgG Avidity ELISA kit (VCA)

For the qualitative determination of Epstein-Barr virus viral capsid (VCA)-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

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

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1. Overview

The Anti-Epstein-Barr Virus IgG ELISA (Enzyme-Linked Immunosorbent Assay) kit (VCA) (ab222940) is designed for the qualitative determination of Epstein-Barr virus viral capsid (VCA)-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

Microplates are coated with specific antigens to bind the corresponding antibodies of the sample (dual pipetting). After washing the wells to remove all unbound sample material, one well is incubated with reagent and the corresponding well with washing buffer. The reagent removes the low-avidity antibodies from the antigens whereas the high-avidity ones are still bound to the specific antigens. After a second washing step to remove the rest of reagent and low-avidity antibodies, a horseradish peroxidase (HRP) labeled conjugate is added. This conjugate binds to the captured antibodies. In a third washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color.

Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

The presence of IgG antibodies to Epstein-Barr Virus indicates the occurrence of the infection but does not distinguish between recent and past infection. Specific IgM antibodies are first detected approximately in ten days and peak at about four weeks post infection. They may persist for several months after acute infections. Based on the evidence that antibody avidity gradually increases after exposure to an immunogen, avidity of IgG antibodies can be used as a marker for distinguishing recent primary from long-term infections. Avidity describes the binding strength of a specific antibody to its antigen. Low-avidity IgG antibodies indicate a primary infection, whereas the presence of IgG antibodies with high avidity points to persistency or reactivation of infection.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add samples, standards and controls to wells used.

Add prepared labeled HRP-Conjugate to each well. Incubate at 37°C.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used to produce 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

  Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

6. Materials Supplied

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Reagent	15 mL	4°C	4°C
Epstein-Barr Virus (VCA) IgG Control Low	2 mL	4°C	4°C
Epstein-Barr Virus (VCA) IgG Control High	2 mL	4°C	4°C
Bottle	1 Unit	4°C	4°C
Epstein Barr virus (IgG) Coated Microplate (12 x 8 wells)	96 Wells	4°C	4°C
IgG Sample Diluent***	100 mL	4°C	4°C
Stop Solution	15 mL	4°C	4°C
20X Washing Solution*	50 mL	4°C	4°C
Epstein Barr virus anti-IgG HRP Conjugate**	20 mL	4°C	4°C
TMB Substrate Solution	15 mL	4°C	4°C
Epstein Barr virus IgG Positive Control***	2 mL	4°C	4°C
Epstein Barr virus IgG Cut-off Control***	3 mL	4°C	4°C
Epstein Barr virus IgG Negative Control***	2 mL	4°C	4°C

* Contains 0.1 % Bronidox L after dilution

** Contains 0.2 % Bronidox L

*** Contains 0.1 % Kathon

7. Materials Required, Not Supplied

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

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 µL and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

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
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- 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 to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Reagent:

Ready to use. Store at 4°C. If crystals have formed in the reagent warm up to 37°C e.g. in a water bath and mix gently until they disappear.

9.2 Epstein-Barr Virus (VCA) IgG Control Low:

1 bottle containing 2 mL control (human serum or plasma); colored yellow; ready to use and stored at 4°C.

9.3 Epstein-Barr Virus (VCA) IgG Control High:

1 bottle containing 2 mL control (human serum or plasma); colored yellow; ready to use and stored at 4°C.

9.4 Bottle:

Empty bottle for ready to use Washing Buffer.

9.5 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 Preparation

- Use Epstein-Barr Virus (VCA) IgG positive human serum or plasma (citrate, heparin) (OD > Cut-off) with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 4°C; otherwise they should be aliquoted and stored deep-frozen (-70 to -20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
- Heat inactivation of samples is not recommended.
- Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10 µL sample and 1 mL IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

11. Assay Procedure

- Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described.
- The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems, we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects.
- Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.
- Perform all assay steps in the order given and without any delays.
- A clean, disposable tip should be used for dispensing each standard/control and sample.
- Adjust the incubator to 37 ± 1 °C.
- For avidity determination dual pipetting of standards/controls and diluted samples is needed.

11.1 Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave wells A1/A2 for the Substrate Blank.

11.2 Cover wells with the foil supplied in the kit.
11.3 Incubate for 1 hour ± 5 min at 37 ± 1 °C.
11.4 When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
ΔNote: Washing is important! Insufficient washing results in poor precision and false results.
11.5 Dispense 100 µL of Reagent in wells B1, C1, D1, E1 etc., except for the Substrate Blank well A1. Dispense 100 µL of Washing Buffer in wells B2, C2, D2, E2 etc., except for the Substrate Blank well A2.
11.6 Incubate for exactly 10 min at 37 ± 1 °C.
11.7 Repeat step 11.4.
11.8 Dispense 100 µL HRP Conjugate into all wells except in the blank wells (A1/A2).
11.9 Incubate for 30 min at room temperature (20 to 25 °C). Do not expose to direct sunlight.
11.10 Repeat step 11.4.
11.11 Dispense 100 µL TMB Substrate Solution into all wells.
11.12 Incubate for exactly 15 min at room temperature (20 to 25 °C) in the dark. A blue color occurs due to an enzymatic reaction.
11.13 Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a color change from blue to yellow occurs.
11.14 Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

12. Calculations

Adjust the ELISA Microwell Plate Reader to zero using the Substrate Blank.

If the ELISA Microwell Plate Reader cannot be adjusted to zero using the Substrate Blank, subtract the absorbance value from all other absorbance values measured to obtain reliable results.

Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

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

Run Validation Criteria

- Substrate Blank: Absorbance value < 0.100
- Control Low: Avidity (%) < 45 %
- Control High: Avidity (%) > 55 %

Calculation of Results

For each sample or control calculate the ratio between the absorbance of the well dispensed with Reagent and the absorbance of the well dispensed with Washing Buffer multiplied by 100

$$\frac{Absorbance\ (sample\ or\ control)\ Avidity\ Reagent}{Absorbance\ (sample\ or\ control)\ Washing\ Buffer\ (diluted\ 1+19)}\ x\ 100 = Avidity\ (\%)$$

ΔNote: For samples with high absorbance values (OD > 2.000) appropriate higher dilutions should be used.

Interpretation of Results

Result	Avidity	Interpretation
Low-avidity IgG	< 45 %	An avidity index of less than 45 % indicates a primary infection acquired within the past 2 months.
Equivocal	45 – 55 %	No clinical interpretation can be deduced from an equivocal result. It is recommended to take a second sample within an appropriate period of time (e.g. 2 weeks) and repeat testing. If the result of the repeated test is still equivocal, precise statements regarding the time of infection cannot be made.
High-avidity IgG	> 55 %	The presence of high-avidity IgG indicates a past infection or reinfection.
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. A result of high avidity cannot exclude the possibility of a recent infection.		

Antibody Isotypes and State of Infection

VCA IgG	VCA IgM	VCA IgG-Avidity	Probable result
+	-	low	Vague, further investigation necessary
+	-	high	Indicatives of a past infection
+	+	low	Suggests a current or very recent infection
+	+	high	Suggests a past infection with persisting IgM or reactivation of infection

13. Performance Characteristics

The evaluation of the performance of the Anti-Epstein-Barr Virus (VCA) IgG assay was performed in comparison to well defined samples. The resulting relative agreement was 99.2 %.

14. Troubleshooting

Problem	Cause	Solution
Low signal	Incubation time to short	Repeat
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type	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)
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

15. Interferences

– Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)