

Anti Epstein Barr Virus Antibody IgM GENLISA® ELISA

REF : KBD105

Ver 1.0

IVD

Enzyme Immunoassay for the Qualitative Determination of Anti
Epstein Barr Virus Antibody IgM in human serum and plasma

IVD	For In-vitro Diagnostic Use	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBD105 96 tests

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Introduction:

Epstein-Barr virus (EBV), also known as human herpesvirus 4, is the causative agent of diseases such as infectious mononucleosis (IM), Burkitt's lymphoma (BL), nasopharyngeal carcinoma.

EBV acquired in early childhood usually does not cause symptoms. Infection in adolescence or early adulthood often results in clinical IM. Typically, the disease presents as pharyngitis, lymphadenopathy, and fever. In most cases, symptoms resolve within 2–4 weeks, but more than 90% of adults develop a latent B-lymphocyte infection. Approximately 1% of immunocompetent individuals may experience severe complications (hepatitis, myocarditis, splenic rupture, neurological complications). In immunosuppressed individuals, primary EBV infection leads to severe disorders (eg, BL).

For laboratory diagnosis of EBV infection, a polymerase chain reaction and serological methods are used that include a test for the detection of heterophilic antibodies and the detection of specific antibodies by ELISA. The latter method allows not only to establish the fact of infection with EBV, but also the stage of the disease.

The optimal combination of serological tests for the diagnosis of EBV infection involves the detection of IgG and IgM antibodies that are specific for the viral capsid antigen (anti-VCA-IgG and anti-VCA-IgM) and nuclear antigen (anti-EBNA-IgG). Anti-VCA-IgM antibodies appear in the body during an early infection and disappear within 4-12 weeks. IgG to VCA appear later, their concentration reaches a maximum level at the initial stages of the disease, gradually decreasing. However, these antibodies are determined throughout life. If antibodies to the viral capsid antigen do not appear, a person is susceptible to EBV infection.

Anti-EBNA-IgG are produced by the body 2-4 months after the onset of symptoms. The concentration of the antibodies is maintained at a high level for a long time or throughout life in the majority of infected people. Simultaneous detection of specific IgG to EBNA and VCA indicates the past infection.

Intended Use:

The Anti Epstein Barr virus Antibody IgM GENLISA® ELISA kit is used as an analytical tool for qualitative determination of Anti Epstein Barr virus Antibody IgM in serum and plasma.

Principle:

The method employs sandwich ELISA technique. Anti IgM antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Anti Epstein Barr virus Antibody IgM present in the sample are bound by the antibodies. Antigen to EBV conjugated to HRP is added and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Anti Epstein Barr virus Antibody IgM in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti IgM Antibody Microtiter Coated Plate (12 x 8 wells) – 1 no
2. Positive Control – 0.5 ml/vial
3. Negative Control – 0.5 ml/vial
4. Sample diluent – 12 ml
5. Epstein Barr virus:Enzyme Conjugate (concentrated) – 1.3 ml
6. Epstein Barr virus:Conjugate Diluent – 13 ml
7. (20X) Wash Buffer – 50 ml
8. TMB Substrate – 12 ml
9. Stop Solution – 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.

7. Absorbent Paper.

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Specimen Collection and Handling:**

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (20X) Dilution: To make Wash Buffer (1X), add 4ml concentrate Wash Buffer (20X) + 76 ml of DI water is sufficient for 8 wells. Once diluted, it is stable at 2-8°C for 7 days. This is the working solution.
3. Enzyme Conjugate Dilution: To make Conjugate (1X), add 100 ul of Concentrated Conjugate to 1 ml of Conjugate Diluent which would be sufficient for 8 wells. Once diluted, it is stable at 2-8°C for 1 day.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **90 ul Sample Diluent** to respective each wells.
3. Add **10 ul of Negative controls, Positive Control and Samples** to respective wells.
4. Cover the plate with a sealer and incubate for 30 minutes at 37°C.
5. Aspirate and wash plate 5 times with diluted Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
6. Add **100 ul of diluted enzyme conjugate** to each well each well.
7. Cover the plate with a sealer and incubate for 30 minutes at 37°C.
8. Aspirate and wash plate 5 times with diluted Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
9. Add **100 ul of TMB Substrate** into each well.
10. Do not cover the plate with a lid and incubate it in the dark for 15 minutes at 18-25°C.

11. Add 100 ul of Stop Solution to all wells. The wells should turn from blue to yellow in color.
12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Validation of the test:

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

Positive Control	OD $\geq$ 1.200
Negative Control	OD $\leq$ 0.150

If one of the negative control absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the other two values. If more than one negative control absorbance does not meet the criteria, the test is invalid and must be repeated.

Calculation of Results:

Calculate the mean absorbance value for 3 negative controls (Nc), Cut off value (CO) and Ratio_{sample}

$$Nc = (Nc1 + Nc2 + Nc3) / 3;$$

$$CO = Nc + 0.3;$$

$$Ratio_{sample} = OD_{sample} / CO,$$

OD_{sample} – optical density of the well containing sample.

Interpretation of Results:

Ratio _{sample} > 1.1	Positive
0.9 $\leq$ Ratio _{sample} $\leq$ 1.1	Equivocal
Ratio _{sample} < 0.9	Negative

If the result is equivocal, repeat the test. If it remains equivocal, collect a new serum sample.

Performance Characteristics:

Sensitivity

The relative sensitivity of Anti Epstein Barr Virus Antibody IgM kit was calculated and determined to be **99.0%**.

Specificity

The relative specificity of Anti Epstein Barr Virus Antibody IgM kit was calculated and determined to be **99.2%**.

Precision

Intra-Assay precision:

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

Serum No	OD	Ratio	CV%
1100	1.468	4.42	6.9
1843	0.753	2.27	6.0

Inter-Assay precision:

Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No	OD	Ratio	CV%
1100	1.533	4.65	6.4
1843	0.806	2.44	6.1

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed.
- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

**LIMITED WARRANTY**

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