Product information

Instruction Manual

EBV EBNA-1 IgG ELISA

Enzyme immunoassay for the detection
of human IgG antibodies against **EBV EBNA-1**
in serum and plasma

Cat. No.: IB79227
Storage: 2-8°C

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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1. Intended Use

The IBL-AMERICA EBV EBNA-1 IgG Antibody ELISA Test Kit has been designed for the detection of IgG class antibodies against EBV EBNA-1 in serum and plasma. For research use only, not for use in diagnostic procedures.

2. Discussion

In 1961 an infectious disease was identified in Uganda, and was named Burkitt lymphoma after its discoverer. In 1964, Epstein, Barr and Achong characterized by electron microscopy as the causing agent a hitherto unknown virus, which belongs to the family of herpes viruses.

3. Principle of the Test

The IBL-AMERICA EBV EBNA-1 IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). EBV EBNA-1 antigen is bound on the surface of the microtiter strips. Diluted unknowns are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized EBV EBNA-1 antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm.

4. Limitations, Precautions and General Comments

- This assay is intended for research use only – not for use in diagnostic procedures.
- Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, they should not be mixed among one another.
- All reagents have to be used within the expiry period.
- In accordance with Good Laboratory Practices (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

- This assay is manufactured for IBL-America.

5. Reagents Provided

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.

Components	Volume / Qty.
EBV EBNA-1 antigen coated microtiter strips	12 x 8 wells
Calibrator A (Negative Control)	1 x 2 mL
Calibrator B (Cut-Off Standard)	1 x 2 mL
Calibrator C (Weak Positive Control)	1 x 2 mL
Calibrator D (Positive Control)	1 x 2 mL
Enzyme Conjugate	1 x 15 mL
Substrate	1 x 15 mL
Stop Solution	1 x 15 mL
Sample Diluent	1 x 60 mL
Washing Buffer (10×)	1 x 60 mL
Plastic bag	1 ea.

5.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with a EBV EBNA-1 antigen (Affinity-purified recombinant EBNA-1 (sf-9/ Baculovirus)). Ready-to-use.

5.2. Calibrator A (Negative Control)
2 mL, protein solution diluted with PBS, contains no IgG antibodies against EBV EBNA-1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.3. Calibrator B (Cut-Off Standard)
2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against EBV EBNA-1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.4. Calibrator C (Weak Positive Control)
2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against EBV EBNA-1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.5. Calibrator D (Positive Control)
2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against EBV EBNA-1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.6. Enzyme Conjugate
15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Ready-to-use.

5.7. Substrate
15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.8. Stop Solution
15 mL, 0.5 M sulfuric acid. Ready-to-use.

5.9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.10. Wash Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.11. Plastic Bag
Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for unknown dilution
- Bidistilled water
- Re-usable black lid for covering
  (Available upon request at IBL-America)

7. Collection and Handling of Unknowns

Principally serum or plasma (EDTA, heparin) can be used in this test. Serum is separated from whole blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. They can be stored refrigerated (4-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. Unknowns should not be frozen and thawed repeatedly. Lipemia, hemolysis or bacterial contamination can cause inaccurate results.

All unknowns must be diluted 1:101 with ready-to-use diluent (e.g. 5 µL serum + 500 µL diluent). No other components of the kit require dilution.

8. Assay Procedure

8.1. Preparation of Reagents

Wash Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and unknowns must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- All test materials should be assayed in duplicate.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for all materials in duplicate and also include a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) unknowns and the **ready-to-use** calibrators and controls respectively into the wells. Leave the substrate blank empty.
3. Cover plate with the re-usable plate cover and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted wash solution. This procedure is repeated for a total of three times. Remove residual wash buffer by inverting the microtiter plate and tapping briskly on absorbent material.
5. Immediately pipet 100 µL of ready-to-use conjugate into the microwells. Leave the substrate blank empty.
6. Cover plate with the re-usable plate cover and incubate at room temperature for 30 minutes.

7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted wash solution. This procedure is repeated for a total of three times. Remove residual wash buffer by inverting the microtiter plate and tapping briskly on absorbent material.
8. Pipet 100 µL of the ready-to-use substrate into all microwells, including the substrate blank.
9. Cover plate with the re-usable plate cover and incubate at room temperature for 20 minutes.
10. To terminate the substrate reaction, pipet 100 µL of the ready-to-use stop solution into all microwells, including the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. Results

The OD of the calibrators (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.

The initial dilution of unknowns has been taken into consideration when reading the results from the graph. Results of unknowns of higher predilution have to be adjusted for the dilution factor.

Unknowns showing concentrations above the highest calibrator have to be diluted as described in “Assay Procedure” (chapter 8.) and reassayed.

Values suggested by literature:

U/mL	
< 8	negative
8 - 12	equivocal
> 12	positive