Epstein Barr Virus EBNA-1 IgG ELISA Kit

Catalog Number KA1448

96 assays

Version: 12

Intended for research use only

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Introduction

Intended Use

Epstein Barr Virus EBNA-1 IgG ELISA Kit is an ELISA kit for the qualitative, semiquantitative and quantitative detection of IgG antibodies against Epstein-Barr virus (EBV) nuclear antigen-1 (EBNA-1) in human serum and plasma.

Background

For detection of EBV-induced or –associated diseases, such as infectious mononucleosis, chronic active EBV infection, Burkitt's lymphoma, carcinomas of Waldayer's ring, opportunistic lymphomas (oligo- or polyclonal) and nasopharyngeal carcinoma. The kit may also be used for the overall characterization of the chronic fatigue syndrome and of immunodeficiency during which EBV is frequently activated.

Principle of the Assay

The Epstein Barr Virus EBNA-1 IgG ELISA Kit is a solid-phase immunoanalytical test. A specific recombinant antigen of EBNA-1 EBV is bound to the surface of the wells. If relevant antibodies are present in the test samples, they bind to the immobilized antigens. In the next step, the bound antibodies then react with horseradish peroxidase-labeled anti-human IgG antibodies. The amount of bound labeled antibodies is determined by a color enzymatic reaction. Negative samples do not react, a slight change in the color of the wells is the background of the reaction.

General Information

Materials Supplied

List of component

Component	Amount
ELISA break-away strips in the handling frame coated with the specific antigen.	96 (8x12) wells
Standard A, negative control human serum, r.t.u.[1), 2)]	1.3 mL
Standard B, r.t.u.	1.3 mL
Standard C, r.t.u.	1.3 mL
Standard D (Calibrator (human serum)), r.t.u.	1.3 mL
Standard E (Positive control human serum), r.t.u.	1.3 mL
Anti-human IgG antibodies labelled with horseradish peroxidase (anti-IgG Px-conjugate), r.t.u.	13 mL
Wash buffer concentrate, 10x concentrated	55 mL
Dilution buffer, r.t.u.	60 mL
Chromogenic substrate TMB, r.t.u.	13 mL
Stop solution, r.t.u.	13 mL

[1)] r.t.u. = ready to use

[2)] The antibody concentration for each Standard (A-E) is mentioned in enclosed Quality control certificate (AU/mL - Artificial units/mL).

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Storage Instruction

- ✓ Store the kit and the kit reagents at 2 to 10°C, in a dry place and protected from the light.
- ✓ Store unused strips back in the package and seal or close tightly in a zippered bag with desiccant.
- ✓ Store unused test samples undiluted, aliquoted and frozen at -18°C to -28°C. Frequent freezing and thawing is not recommended. If you store samples at +2°C to +10°C, then test them within one week.
- ✓ Test sample solutions at the working concentration cannot be stored. Always prepare them fresh.

Materials Required but Not Supplied

- ✓ Distilled or deionised water for dilution of the Wash buffer concentrate (10x).
- ✓ Appropriate equipment for pipetting, liquid dispensing and washing.
- ✓ Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).
- ✓ Thermostat (set at 37°C) for ELISA plate incubation.

Precautions for Use

✓ Safety Precautions

- All ingredients of the kit are intended for laboratory use only.
- The manufacturer guarantees the usability of the kit as a whole.
- Wash buffer (10x), chromogenic substrate, stop solution, and dilution buffer are interchangeable between the kits, unless otherwise noted in the kit instructions.
- Work aseptically to avoid microbial contamination of samples and reagents.
- When collecting, diluting, and storing reagents, be careful not to cross-contaminate them or contaminate them with enzymatic activity inhibitors.
- The chromogenic substrate shouldn't come into contact with oxidizing agents and metal surfaces. Because it is sensitive to light, close the bottle immediately after use. The chromogenic substrate must be clear in use. Do not use the solution if it is blue.
- Follow the instruction manual exactly. Non-reproducible results may arise in particular:
  - Insufficient mixing of reagents and samples before use.
  - Inaccurate pipetting and non-compliance with the incubation times.
  - Poor washing technique and splashing of the edges of the wells with sample or conjugate.
  - Using the same tip when pipetting different solutions or swapping caps.
- Human control sera and standards used in the kit were tested for the absence of HBsAg, HCV and anti-HIV-1,2 antibodies. Treat test specimens, control sera, standards, and use strips as infectious material. Autoclave items have been in contact with them for 1 hour at 121°C or disinfect for at least 30 minutes with 3% chloramine solution.
- Neutralize liquid waste containing stop solution (sulfuric acid solution) with 4% sodium bicarbonate solution before disposal.
- Disinfect the waste generated during strip washing in a waste container using a suitable disinfectant solution (eg Incidur, Incidin, chloramine,) at the concentration recommended by the manufacturer.
- Handle stop solution carefully to avoid splashing on the skin or mucous membranes. If this happens, wash the affected area with plenty of running water.
- Do not eat, drink, or smoke while working. Do not pipette by mouth, but by suitable pipetting devices. Wear protective gloves and wash your hands thoroughly after work. Be careful not to spill specimens or form an aerosol.
- All reagents and packaging material must be disposed of in accordance with applicable legislation.
- In case of suspicion of an adverse event in connection with the use of the kit, inform the manufacturer and the competent state authority without delay.

Assay Protocol

Reagent and Sample Preparation

- ✓ Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.
- ✓ Thoroughly mix Dilution buffer, conjugate anti-IgG Px and Chromogenic substrate.
- ✓ Thoroughly mix test samples and control sera just prior to testing. Dilute the test samples 101x with Dilution buffer (eg. 5 µL of serum sample + 500 µL of Dilution buffer). Do not dilute control sera and calibrator, they are in working concentration (r.t.u ready to use).
- ✓ Prepare a working concentration of wash buffer (10x) by diluting it 10x in a suitable volume of distilled/deionized water (eg. 50 mL of Wash buffer (10x) + 450 mL of distilled water). If there are salt crystals in the concentrated solution, warm it in a water bath of +32°C to +37°C and mix well before diluting. Unused wash solution in working concentration can be stored for 1 month at room temperature.
- ✓ Do not dilute conjugate anti-IgG Px, chromogenic substrate, stop solution, they are ready to use.

Assay Procedure

Manufacturer will not be held responsible for results if manual is not followed exactly.

1. Allow the microwell strips sealed with desiccant, to reach room temperature before opening the bag, to avoid dew condensation of the plate. Prepare the required number of strips for the reaction. Seal unused strips together with the desiccant in a zipper bag or seal under vacuum.
2. Choose the proper method for data interpretation (see below and/or Data analysis) and pipette Standards and samples according to the Plate Layout. Start with filling the first well with 100 µL of Dilution buffer to estimate the reaction background. In case of choosing the qualitative or semiquantitative method, fill two wells with 100 µL/well of Calibrator (Standard D), next well with Positive control serum (Standard E) and next one well with 100 µL of Negative control serum (Standard A) (see Qualitative and semiquantitative method in Plate Layout).

   In case of quantitative method, pipette 100 µL all Standards A-E (see Quantitative method in Plate Layout). Fill the remaining wells with 100 µL of diluted serum samples (S1, S2, S3, ...). It is sufficient to apply samples as singlets, however, if you wish to minimize the laboratory error apply control sera and tested samples as doublets, Standard D as triplet. We recommend to include positive reference serum sample (your in-house internal control) into each run to follow the sequence, variability and accuracy of calibration.
3. Incubate 30 minutes (±2 min) at 37°C.
4. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µL/well of Wash buffer. Avoid over flowing the solution out of the wells. Aspirate the contents of the wells and tap the plate on an adsorbent paper.
5. Mix thoroughly the vial of anti-IgG Px conjugate and pipetter 100 µL of anti-IgG Px conjugate into the

wells. Incubate 30 minutes (±2 min) at 37°C.

6. Aspirate the fluid from the wells and wash them four times with 250 µL/well of Wash buffer.
7. Dispense 100 µL of TMB substrate into each well; pipette in a regular rhythm or use an appropriate dispensing instrument.
8. Incubate for 15 minutes (+/-30 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminum foil or keep them in the dark during the incubation with TMB substrate.
9. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate few times to ensure complete mixing of the reagents.
10. Measure the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 620-690 nm.

✓ Flow Chart

Step 1.	Prepare reagents and test samples in working concentration
	↓
Step 2.	Apply 100 µL/well of control sera and test samples
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 3.	Apply 100 µL/well of anti-IgG Px-conjugate r.t.u.
	↓
	Incubate 30 minutes at 37°C
	↓
	Wash 4 times (250 µL/well), aspirate
	↓
Step 4.	Apply 100 µL/well of Chromogenic substrate TMB
	↓
	Incubate 15 minutes in dark at room temperature
	↓
Step 5.	Dispense 100 µL/well of Stop solution
	↓
Step 6.	Read the absorbance at 450/ 620-690 nm within 20 minutes

Data Analysis

Calculation of Results

✓ Processing of Results

First, subtract the absorbance of the well with dilution buffer from the calibrator, control sera, and test samples.

If the values of control sera or tested samples are negative after background subtraction, consider them as zero value.

- Processing of results for Qualitative interpretation

1. Calculate the mean OD value of the standard D from the two wells. If you are applying three standard D wells and some of these values differ by more than 20% from the mean, don not use it for calculation and calculate the mean of the remaining two values.
2. Determine the cut-off value by multiplying the mean OD value of the standard by the correction factor. The value of the correction factor is stated in the QC certificate for the given lot no.
3. Samples with an OD value < 90% cut-off are negative and samples with an OD value > 100% cut-ff are considered positive.

- Processing of results for Semiquantitative interpretation

Determine Positivity Index for each sample:

1. Compute the cut-off value (see the previous paragraph)
2. Determine the index value for each sample by dividing the OD of the test sample by the cut-off value.
3. Read the appropriate degree of reactivity of the sample. See below:

Positivity index	Evaluation
< 0.90	Negative
0.90 – 1.10	+/-
>1.10	Positive (on the basis of the positivity index value it is possible to estimate semi quantitatively the amount of antibodies in the sample.)

Example of calculation:

Standard D absorbances = 1.023; 1.101

Standard D mean absorbance = 1.062

Sample absorbance = 0.800

Correction factor = 0.18

Cut-off value = 1.062 x 0.18 = 0.191

Sample Positivity Index = 0.800 / 0.191 = 4.19

Note: a rating of +/- means that the sample is in the gray zone. Repeat the test for this result. If the sample is again in the gray zone after retesting, repeat the test with an alternative method or use a sample from a new

sample from the same individual.

✓ Processing of results for Quantitative interpretation

Compute the sample antibody titers in artificial units (AU/mL) as follows:

1. Construct a calibration curve by plotting the units of Standards (x-axis) to absorbance of Standard (y-axis). We recommended using logarithmic x-axis. The concentration of each Standard (A-E) is mentioned in enclosed Quality control certificate.
2. Find the place where the absorbance of tested samples intersect calibration curve and find the corresponding values (AU/mL) on the axis x. It is possible to use various softwares for the standard curve fitting and for calculation of the unknowns, e.g. Winliana, KimQ. For better fitting, the polynomic (four-parameter) function is the most convenient.

The evaluation in arbitrary units for sera is stated in the Quality Control Certificate.

Note1 : a rating of +/- means that the sample is in the gray zone. Repeat the test for this result. If the sample is again in the gray zone after retesting, repeat the test with an alternative method or use a sample from a new sample from the same individual.

Note2: quantification is accurate only in the linear part of the calibration curve. If the measured OD of the sample exceeds the linearity interval (OD 0.100-2.500), it is necessary to repeat the testing of the sample at a higher dilution for accurate quantification.

✓ Interpretation of Results

Interpretation of Results - Antibodies							
VCA EBV			EA(D) EBV		EBNA-1 EBV		
IgG	IgM	IgA	IgG	IgM	IgG	IgM	Stages of EBV infection
-	-	-	-	-	-	-	Seronegative
-	+	+	-	+ or -	-	+	Primoinfection (early phase)
+ low avidity	+	+	+ or -	+ or -	-	+	Primoinfection
	+	-	+ or -	+ or -	-	+	
	-	+	+ or -	+ or -	-	-	
+ high avidity	+	-	+ or -	-	+	-	Suspect reactivation
	-	+	+ or -	-	+	-	
	-	-	++	-	+	-	
+ high avidity	-	-	-	-	+	-	Seropositivity without symptoms of active infection

Explanatory notes:

+ serum evaluated as positive

- serum evaluated as negative

Anomalous result of finding positive levels in both IgG and IgM anti-EBNA-1 antibody classes can be a sign of an autoimmune disease.

Performance Characteristics

✓ Validity of the test

The test is valid if:

- The background absorbance (the absorbance of the Dilution buffer) is less than 0.250.
- The mean absorbance values of standards/control sera, and the ratio between the absorbance values of Standard E/Standard D are in the ranges stated in the Quality control certificate for this kit lot.

✓ Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different absorbance values.

- Intraassay variability

  The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot at least on 12 parallels of the same microtitration plate.

  Example: (N = number of parallels of the same microtitration plate, ± σ = standard deviation)

N	A	± σ	CV%
16	0.614	0.021	3.4%
16	1.566	0.060	3.9%

- Interassay variability

  The coefficient of intraassay variability is max. 15%. It is measured for each lot as comparison of the OD values of the same serum sample in several consecutive tests.

  Example: (N = number of an independent examination of the same serum sample, ±σ = standard deviation)

N	A	± σ	min-max	CV%
16	0.618	0.059	0.513-0.744	9.5%
17	1.071	0.072	0.965-1.214	6.9%
16	2.454	0.096	2.306-2.630	3.9%
16	1.404	0.062	1.303-1.501	4.4%

✓ Recovery test

Measured values of recovery test for every Lot are between 80-120% of expected values.

- ✓ Sensitivity and specificity

  Sensitivity of the test is 100%. Evaluation was performed on blood samples tested with another commercially available test. Samples were expected to be positive for anti-EBNA-1 EBV IgG antibodies (blood donors, persons with history of infectious mononucleosis).

  Specificity of the test is 96.4%. Specificity was determined on blood samples from healthy EBV negative blood donors.

- ✓ Analytical sensitivity of the test

  The analytical sensitivity of the assay is defined as the mean of the sample without analyte plus three times of the standard deviation and represents the lowest detectable antibody titer. The analytical sensitivity value is determined for each kit lot and is stated in the QC certificate of that kit lot.

- ✓ Analytical specificity of the test

  The quality of the specific recombinant EBNA-1 epitope specific antigen used, which recognizes specific antibodies in samples, ensures high specificity and sensitivity. However, to determine the diagnosis, the test results must always be interpreted in the context of results of other lab tests, see interpretation of results.

- ✓ Measuring range

  the measuring range is determined by the measuring capability of the spectrophotometer/colorimeter used.

- ✓ Interference

  Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 50 mg/mL of triglycerides. Nevertheless, such samples can only be tested with reservations.

- ✓ Limit of quantification

  The limit of quantification is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence. This value is determined for each batch of the kit and is stated in the QC certificate of the given batch of the kit.

Resources

References

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4. Smith R.S. *et al.*: A synthetic peptide for detections antibodies to Epstein-Barr virus nuclear antigen in sera from patients with infectious mononucleosis, *J. Infect. Dis.* **154**: 885–889, 1986.
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Plate Layout

Qualitative and semiquantitative evaluation

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution buffer											
B	Standard D											
C	Standard D											
D	Standard E											
E	Standard A											
F	Sample 1											
G	Sample 2											
H	Sample 3											

Quantitative evaluation after one well

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution buffer	Sample 3										
B	Standard A	Sample…										
C	Standard B											
D	Standard C											
E	Standard D											
F	Standard E											
G	Sample 1											
H	Sample 2											

Quantitative evaluation after two well

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution buffer	Sample 2										
B	Standard A	Sample 2										
C	Standard B	Sample …										
D	Standard C	Sample …										
E	Standard D											
F	Standard E											
G	Sample 1											
H	Sample 1											