

**REF**

GD7780 00

EBV-EBNA IgG

Enzyme immunoassay for the quantitative determination of IgG antibodies to Epstein Barr Virus nuclear antigen or EBV EBNA in serum or plasma

**IVD**

INDICATION

Epstein-Barr virus (EBV) is the principal etiological agent of infectious mononucleosis, as well as a contributory factor in the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma (NPC). It is a member of the Herpesviridae family, and has a worldwide distribution, such that 80-90% of all adults have been infected. Primary infections usually occur during the first decade of life. While childhood infections are mostly asymptomatic, 50-70% of young adults undergoing primary EBV infections show mild to severe illness. EBV may cause a persistent, latent infection, which can be reactivated under immunosuppression or in AIDS affected patients.

As humoral responses to primary EBV infections are quite rapid, the level and class of antibodies raised in most cases allow classification as to whether the patient is still susceptible, has a current or recent primary infection, had a past infection or may be having reactivated EBV infection. The detection of EBV-specific IgG, IgM and IgA antibodies has become therefore an important and useful determination for the monitoring and the follow-up of EBV infected patients.

PRINCIPLE OF THE ASSAY

Microplates are coated with synthetic peptides derived from immunodominant specific sequences relative to nuclear antigen EBNA-1, which does not present any homology with other Herpesviridae.

The solid phase is first treated with diluted sample. After a washing step, the IgG, specifically bound to antigens, are detected with anti-human IgG antibodies conjugated to horseradish peroxidase (HRP). A substrate/chromogen solution is added and the intensity of the generated colour is proportional to the amount of anti-EBV EBNA IgG antibodies present in the specimen.

IgG concentrations in samples are quantified by means of a standard curve calibrated in arbitrary unit per milliliter (arbU/ml), as no international standard is available.

KIT CONTENT

1. Reagent A – Microplate

12x8 strips.

8 wells breakable strips, coated with EBV-EBNA-1 specific synthetic peptides. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. Reagent B1 – Enzymatic Tracer 20x

1 vial of 0.8 ml.

Stabilized proteic buffer solution containing human anti-IgG polyclonal antibody conjugated with Horseradish peroxidase (HRP), 20x concentrated.

3. Reagent B2 – Tracer Diluent

1 vial of 16 ml.

Proteic buffer solution for the dilution of the concentrated tracer; it contains 0.02 % Gentamicin sulphate and 0.3% Kathon GC as preservatives.

4. Reagent C – Washing Solution 20x

1 vial of 60 ml.

Concentrated solution to be diluted 1:20 with distilled water. It contains PBS buffer pH 7.4 with detergents and 0.1% Kathon as preservative.

5. Reagent D – Chromogen

1 vial of 8 ml.

Ready to use solution containing, in phosphate/citrate buffer, Tetramethylbenzidine (TMB) with activators and stabilizers.

Avoid light exposure.

6. Reagent E – Substrate

1 vial of 8 ml.

Ready to use solution containing Urea peroxide in phosphate/citrate buffer.

7. Reagent F – Stop Solution

1 vial of 16 ml.

Ready to use solution, it contains a mixture of 1 M Hydrochloric and Phosphoric acid.

The reagent is **irritant**: x_i R36/37/38; S(1/2)26-45 Handle with care.

8. Reagent G – Sample Diluent

1 vial of 100 ml.

Proteic solution for sample preparation; it contains proteic stabilizers, 0.09% Sodium azide and 0.3% Kathon GC as preservatives.

9. EBNA IgG Standards

6 vials of 2 ml each.

Ready to use liquids containing EBNA IgG at the following concentrations:

S₀: 0 arbU/ml, **S₁**: 5 arbU/ml, **S₂**: 10 arbU/ml,

S₃: 20 arbU/ml, **S₄**: 50 arbU/ml, **S₅**: 100 arbU/ml.

Standards are prediluted in buffer containing proteic stabilizers, 0.09% Sodium azide and 0.3% Kathon GC as preservatives.

10. Cardboard sealers

2 cardboard sealers to be used to cover the plate during the incubations.

11. Package insert: instruction for use GD7780 00 it/ing.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HBsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples. All objects come in direct contact with samples and all residuals of the assay should be treated or eliminated as potentially infected. Best procedures for inactivation are treatments with autoclave at 121 °C for 30 minutes or with sodium hypochlorite at a final concentration of 2.5% for 24 hours.
2. Avoid any contact with skin and mucous membrane, in particular for Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported in "WASHING INSTRUCTION".
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.

STORAGE AND STABILITY

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before re-store at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. The diluted washing solution can be stored for one week at room temperature or 3 weeks at 2-8 °C.
4. Diluted tracer is stable one week at 2-8 °C, if stored in a disposable sterile container.
5. When preparing chromogen/substrate we recommend the use of plastic disposable containers. The chromogen/substrate solution is stable for 4 hours at room temperature, protected from light.
6. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes of 10, 200 and 1000 µl
- Vortex mixer and absorbent paper
- Chronometer
- Ultrapure Elisa grade water
- Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filters of 450 nm and 620-630 nm.
- Microplate incubator set at 37 (±1) °C.
- Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

SAMPLES

Either serum or plasma can be used. If the assay is not immediately performed, the samples should be kept at 2-8 °C for one week; otherwise they should be stored at - 20 °C. Avoid repeated freeze-thaw cycles. Samples must not be turbid, lipemic, haemolyzed and microbiologically contaminated.

REAGENTS PREPARATION

- **WASHING SOLUTION:** dilute 1:20 with distilled or ELISA grade water (e.g. 60 ml of Reagent C + 1200 ml of distilled water) and mix carefully before use. It is recommended to store diluted washing solution at room temperature for immediate use.
- **TRACER:** dilute concentrated tracer (Reagent B1) 1:20 with Tracer Diluent (Reagent B2) and mix carefully on vortex.
- **CHROMOGEN/SUBSTRATE:** prepare in disposable plastic container, according to needs, the substrate/chromogen solution by mixing Reagent D with Reagent E in equal volumes.

WASHING INSTRUCTION

A good washing procedure is essential to obtain correct and precise analytical results.

We therefore recommend to use a good quality ELISA microplate washer, maintained at a good level of washing mechanical performances.

Generally, 3-5 automatic washing cycles of 0.3 ml/well are sufficient to avoid false positive reactions and remove high background. Anyhow we recommend to calibrate the washing system on the kit itself so to match the declared analytical performances.

In case of manual washing, we suggest to perform 5 washing cycles, dispensing and aspirating 0.3 ml/well per cycle.

In any case the liquid washed out from the plates must be inactivated with a sodium hypochlorite solution at a final concentration of 2.5%, before being thrown away or autoclaved, as it must be considered as potentially infected.

ASSAY PROCEDURE

1. At least one hour before use, bring all reagents, standards and samples to room temperature (18-30 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute standards and samples in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. The colour developed in the last incubation is stable for a maximum of one hour. Otherwise, in case of reading after 10-15 min after dispensing stop solution, immediately place the strips **in the dark**.
8. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-630 nm and to read the absorbance of samples and standards at 450 nm. The "blanking" of the instrument is to be carried out in the blank reagent well where only substrate-chromogen and stop solutions are added.

ASSAY SCHEME

- Dilute samples 1:101 with Sample Diluent (e.g.: 10 µl sample + 1000 µl of Reagent G).
Do not dilute standards. Carefully mix on vortex before dispensing.
- Follow the scheme:

	EBNA-1 synthetic antigens coated wells			
	REAGENTS	Blank	Standard	Sample
First incubation	Standard	-	100 µl	-
	Diluted sample	-	-	100 µl
	- Cover the strips with cardboard sealer - Incubate 60 minutes at 37 (± 1) °C			
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Second incubation	Diluted tracer	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate 60 minutes at 37 (± 1) °C			
Wash	- Peel out the cardboard sealer and aspirate the reaction solution from all wells - Rinse 5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid			
Colorimetric reaction	Chromogen/Substrate (Reagents D+E)	100 µl	100 µl	100 µl
	- Cover the strips with cardboard sealer - Incubate 20 minutes at room temperature (20-25 °C), avoiding light exposure			
	Reagent F (Stop Solution)	100 µl	100 µl	100 µl
	Read the absorbance of each well against Blank at 450 and 620-630 nm			

VALIDITY OF THE ASSAY

The assay is to be considered valid if:

- The OD 450 nm of the blanking well is lower than 0.100. Higher values indicate a chromogen/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
- After subtracting the blank, the mean OD 450 nm value for the Standard 0 arbU/ml is lower than 0.200. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
- The mean OD 450 nm value of the Standard 5 arbU/ml is higher than the Standard 0 arbU/ml value.
- The mean OD 450 nm value of the Standard 100 arbU/ml is higher than 1.000. Lower values indicate kit or calibrator decay. Before repeating the assay, check the expiry date of the kit.

CALCULATION OF RESULTS

Draw a standard curve by plotting the absorbance of the standards (y axis) with the corresponding concentrations in arbU/ml (x axis) on a lin-lin paper.

Concentrations of the samples are obtained by interpolating the mean absorbance value of each sample on the calibration curve.

In case of automatic calculation we recommend to elaborate the standard curve with the "4 parameters" curve fitting system.

Example of calculation

Do not utilize for the calculation of results.

Standard	arbU/ml	OD 450 nm
S ₀	0	0.061
S ₁	5	0.280
S ₂	10	0.520
S ₃	20	0.972
S ₄	50	1.496
S ₅	100	2.058
Positive sample	62	1.633

RESULTS INTERPRETATION

From the study of a donors population, it has been observed that the Standard 5 arbU/ml can be used to discriminate negative from positive samples:

- Samples with **concentration < 5 arbU/ml** are considered **negative** to anti EBNA IgG antibodies.
- Samples with **concentration > 5 arbU/ml** are considered **positive** to anti EBNA IgG antibodies.
- Samples with **concentration in the range 5-10 arbU/ml** are considered in a "gray zone"; it is suggested to keep the subject under clinical control.

It is recommended each laboratory to establish its appropriate cut-off on the basis of the analyzed population and of other clinical and pathological data.

For a correct and precise Epstein Barr Virus diagnosis, the determination of anti EBNA IgM antibodies and anti capsidic antigens antibodies (EBV-VCA IgM, IgG) in combination are recommended.

Clinical features in EBV infections

- Early acute primary infection phase:
 - presence of VCA IgM
 - absence of VCA IgG
 - possible presence of EBNA IgM
 - absence of EBNA IgG
- Late acute primary infection phase:
 - presence of VCA IgM
 - presence of VCA IgG at low avidity
 - presence of EBNA IgM
 - presence of EBNA IgG
- Convalescence or post-infective phase:
 - absence of VCA IgM
 - presence of VCA IgG at high avidity
 - absence of EBNA IgM
 - presence of EBNA IgG
- Reactivation phase (in immunosuppression or deficiency):
 - presence of VCA IgM with low title
 - presence of VCA IgG at high avidity
 - presence of EBNA IgM with low title
 - presence of EBNA IgG

ANALYTICAL PERFORMANCES

Reproducibility

a. Within Run

Within run precision has been determined on 20 replicates of three different samples in the same analytical run. CV values ranging from 3.8 to 19.7% have been found, depending on OD 450 nm values.

b. Between Run

Between run precision has been determined on replicates of two different samples with three different lots. CV values ranging from 5.4 to 18.4% have been found, depending on OD 450 nm values.

Diagnostic sensitivity and specificity

Diagnostic sensitivity and specificity have been determined on a panel of positive and negative samples in comparison with a reference test.

The following results have been found:

Sensitivity: 96.4%
Specificity: 97.5%

PRECAUTIONS IN USE

Reagent F is irritant (Xi). Refer to Safety Data Sheet.

The other reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

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