

## EBV-EA IgG

**Enzyme Immunoassay for the quantitative  
determination of IgG antibodies to Epstein Barr  
Virus specific Early Antigen or EA  
in serum and plasma**

- For in vitro diagnostic use only -

**96 tests**

**Cod. GD7810 00**

### INTRODUCTION

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. It is a member of the family Herpesviridae and it has such a worldwide distribution that 80 to 90% of all adults have been infected. Primary infections usually occur during the first decade of life.

While childhood infections are mostly asymptomatic, 50 to 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 EBV-specific immunodominant synthetic antigens derived from Early Antigen or EA.

In the first incubation, the solid phase is treated with diluted samples and anti-EA IgG are captured, if present, by the antigens.

After washing out all the other components of the sample, in the second incubation bound anti-EA IgG are detected by the addition of anti hIgG antibody, labelled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti EA IgG antibodies present in the sample. IgG in the sample may be quantitated by means of a standard curve calibrated in arbitrary units per milliliter (Uarb/ml) as no international standard is available.

### CONTENT OF THE KIT AND REAGENTS PREPARATION

1. **Reagent A - Microtiter strips** : 1 microtiter plate.  
12x8-wells strips coated with synthetic EA polypeptides.  
The plate is contained in a sealed bag with desiccant.  
Bring the plate to room temperature **before** use, to prevent any moisture formation inside the bag.  
**Note:** upon first opening, residual strips may be used until the desiccant turns pale green.
2. **Reagent B1 - Enzymatic tracer** : 1 vial of 0.8 ml.  
Proteic buffer solution containing a specific anti-hIgG antibody, conjugated with HRP, 20 x concentrated.  
It contains proteic stabilizers, 0.2 mg/ml gentamicin sulphate and 0.3% Kathon GC as preservatives.  
**Note:** when diluted 20x the enzyme conjugate is not stable.  
Dilute only the volume necessary to the test.
3. **Reagent B2 – Tracer diluent** : 1 vial of 16 ml.  
Proteic buffer solution for the dilution of the concentrated tracer. It contains proteic stabilizers, 0.2 mg/ml gentamicin sulphate and 0.3% Kathon GC as preservatives.
4. **Reagent C - Washing Solution:** 1 vial of 60 ml.  
20x concentrated solution to be diluted up to 1200 ml with bidistilled water.  
It contains a phosphate buffer, Tween 20 and Kathon GC as preservatives. The diluted solution, when stored at room temperature, is stable for at least 1 week.
5. **Reagent D/E - Chromogen /Substrate:** 1 vial of 16 ml.  
The solution contains tetramethylbenzidine (TMB) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) with activators and stabilizers, diluted in phosphate/citrate buffer.  
**Note:** store protected from light as sensitive to strong illumination.
6. **Reagent F - Stop Solution** : 1 vial of 16 ml.  
It contains a solution of 0.3 M H<sub>2</sub>SO<sub>4</sub>.  
**Warning :** avoid contact with eyes or skin. Irritant!
7. **Calibration curve** : 6 vials of 2 ml each.  
The calibration set contains the following ready-to-use standards :  
0 - 5 - 10 - 20 - 50 – 100 Uarb/ml.  
Standards are pre-diluted in the sample diluent.  
**Do not dilute again!**
8. **Reagent G - Sample diluent** : 2 vials of 60 ml.  
Proteic solution for samples preparation; it contains a detergent, proteic stabilizers, 0.1% sodium azide and 0.3% Kathon GC as preservatives.
9. **Cardboard sealers** : 2 cardboard sealers to be used to cover the plate during the incubations.

**Note :** All human serum derived materials have been tested as negative for HBsAg, HCV and HIV antibodies with FDA approved kits.

## CONDITIONS AND NOTICES

1. All the reagents contained in the kit are for in vitro diagnostic use only.
2. Do not use the kit or reagents after the expiry date stated on the labels. Do not mix reagents from different lots.
3. Procedures should be performed carefully, in order to obtain reliable results and clinical interpretations.
4. Bring all reagents to room temperature at least 60 minutes before the analysis.
5. 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.
6. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported at point "WASHING INSTRUCTION". It is recommended anyway to use a good quality microplate washer.
7. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces ; avoid intense light exposure during incubation or reagent preparation.
8. Samples and materials potentially infected have to be handled with care as they could transmit the infection.  
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.
9. Avoid any contact with skin and mucosas, specially with the blocking reagent.
10. In any case, use protective talk-free gloves.

## STORAGE AND STABILITY OF THE KIT

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 dessicant and firmly sealed before re-store at 2-8°C.
3. The diluted washing solution can be stored for one week at room temperature or 3 weeks at +2-8°C.
4. The dissolved tracer is stable for 1 week at 2-8°C, when stored in disposable sterile container.
5. All other liquid reagents are stable when stored at 2-8°C, provided that they are handled carefully to avoid any environment contamination.

## MATERIALS NOT PROVIDED IN THE KIT

1. Semi-automatic pipettes of 10, 100 and 1000 µl.
2. Vortex mixer and absorbent paper.
3. Ultrapure water (bidistilled).
4. Chronometer.
5. Photometric reader of microplates or microstrips, linear up to at least 2 OD and supplied with filters of 450 nm and 620-630 nm.
6. Incubator set at +37°C.
7. Automatic microplates washing device or manual apparatus capable of aspirating and dispensing volumes of 300 µl.

## SAMPLES

Either fresh sera or plasma can be used.

If not used immediately, they can be stored at 2-8°C for 1 week; in case of longer storage, freeze at -30°C.

Samples should be without particles or microbial contaminations; in case, centrifuge at 2000 g for 20 minutes or filtrate with a 0.2µm filter.

Highly lipemic or hemolized samples can give uncorrect analytical results.

## REAGENTS PREPARATION

1. **WASHING SOLUTION** : dilute 1:20 with bidistilled water and mix carefully before use.
2. **TRACER** : dilute 1:20 the concentrated tracer with tracer diluent.  
Mix carefully on vortex.

## 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, 4-5 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

### NOTES

1. At least one hour before use, bring all the components of the kit to room temperature (18-30°C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute the calibrators 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 color developed in the last incubation is stable for a maximum of one hour 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 (well A1).

**ASSAY SCHEME**

1. Dilute the sample 1:101 with the Sample Diluent (Ex.: 10 µl sample + 1000 µl diluent).  
The controls are ready-to-use; do not dilute! Mix carefully on vortex before dispensing.
2. Follow this scheme :

First incubation	REAGENTS	EBV antigens coated wells		
		A1 BLANK	STANDARDS	SAMPLE
First incubation	Standards	-	100 µl	-
	Sample	-	-	100 µl
	<ul style="list-style-type: none"> <li>- Cover strips with adhesive film.</li> <li>- Incubate <b>60 min. at +37°C.</b></li> </ul>			
Washing	<ul style="list-style-type: none"> <li>- Peel out the adhesive film and aspirate the reaction solution from all wells.</li> <li>- Wash 4-5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid.</li> </ul>			
Second incubation	Diluted Tracer	-	100 µl	100 µl
	<ul style="list-style-type: none"> <li>- Cover strips with adhesive film.</li> <li>- Incubate <b>60 min. at +37°C.</b></li> </ul>			
Washing	<ul style="list-style-type: none"> <li>- Peel out the adhesive film and aspirate the reaction solution from all wells.</li> <li>- Wash 4-5 times with 300 µl of diluted washing solution, carefully aspirating off the remaining liquid.</li> </ul>			
Colorimetric reaction	Reagents D/E Chromogen/Substrate	100 µl	100 µl	100 µl
	<ul style="list-style-type: none"> <li>- Cover strips with a new adhesive film.</li> <li>- Incubate <b>20 min. at room temperature</b>, protected from light.</li> </ul>			
	Reagent F (Stop solution)	100 µl	100 µl	100 µl
	Read the absorbance of each well against A1 blanking-well at 450 nm and 620-630 nm.			

**VALIDITY OF THE ASSAY**

The assay is to be considered valid if :

1. 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.
2. After subtracting the blank, the mean OD value of the Standard 0 Uarb/ml standard is lower than 0.200.
3. The OD 450 nm mean value of the Standard 5 Uarb/ml is higher than the one of the Standard 0 Uarb/ml.
4. The OD 450 nm mean value of the positive control or the Standard 100 Uarb/ml is higher than 1.000.

In case data above do not match the correct values, before repeating the test check carefully the expiration date of the kit, the performances of the instruments used for the assay and the procedure of distribution of controls and samples.

**CALCULATION OF RESULTS**

If the test turns out to be valid, elaborate the standard curve with a qualified curve fitting system and then calculate the concentration of samples on the curve. The value of 5 Uarb/ml may be used to discriminate between the IgG negative and positive population.

**Example of Standard Curve**

0 Uarb/ml	0.040 OD450nm
5 Uarb/ml	0.200 OD450nm
10 Uarb/ml	0.350 OD450nm
20 Uarb/ml	0.600 OD450nm
50 Uarb/ml	1.300 OD450nm
100 Uarb/ml	2.000 OD450nm

**Important note: Do not use these data to make real calculations !**

**EXAMPLE OF CALCULATION**

Negative Control mean OD 450 nm	0.050
Positive Control mean OD 450 nm	1.200
Cut-Off = NC + 0.150	0.200

Sample#1 OD 450 nm – 0.080	negative
Sample#2 OD 450 nm – 1.158	positive

**Standard curve**

0	Uarb/ml	0.040	OD 450 nm
5	Uarb/ml	0.210	OD 450 nm
10	Uarb/ml	0.380	OD 450 nm
20	Uarb/ml	0.560	OD 450 nm
40	Uarb/ml	0.900	OD 450 nm
160	Uarb/ml	2.800	OD 450 nm

**ASSAY PERFORMANCES**

**Sensitivity:** the sensitivity of the assay has been calculated on a panel of seroconversion and of positive samples by comparing with a FDA approved kit on the market.  
The test shows a sensitivity > 98%.

**Specificity:** the specificity of the assay has been calculated on panels of negative and positive samples, preclassified with an FDA approved kit present on the market.  
The test shows a specificity > 98% on plasma and sera.


**Riproducibility:** It has been calculated on the Negative and Positive Control tested in replicates in different days. CV's between 4-12% have been obtained depending on their OD 450 nm value.

**REFERENCES**

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Legend of the symbols used on the labels:

 Prodotto conforme alla Direttiva 98/79/CE


 Per uso diagnostico in vitro


 Numero di lotto

 Codice

 Limiti di temperatura per la conservazione

 Data di scadenza (anno – mese)

 Consultare i documenti allegati

 Consultare le istruzioni per l'uso

 Rischio biologico

 Fabbricante

 Taglio

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Manufactured by:



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