

## EBV-EA IgM

**Enzyme immunoassay for the qualitative determination of IgM Class antibodies to Epstein Barr Virus- specific Early Antigen or EA.**

- For in vitro diagnostic use only -

**96 tests**

**Cod. GD7815 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, or NPC. A member of the family Herpesviridae, it has a worldwide distribution, such 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.

The solid phase is first treated with diluted sample.

After the washing steps, the IgM, specifically bound to antigens, are detected with goat anti-human IgM antibodies conjugated to peroxidase. A substrate/chromogen solution is added and the intensity of the generated color is proportional to the amount of anti-EBV EA IgM antibodies in the specimen.

The Neutralizing Reagent contains anti-human IgG blocking antibodies to prevent the assay from interferences due to Rheumatoid Factor and to IgG.

### CONTENT OF THE KIT

1. **Reagent A - Microtiter strips** : 1 microtiter plate.  
12x8-wells strips coated with polypeptides specific of EBV-EA. The plate is contained in a sealed bag with dessicant. Bring the plate to room temperature **before** use, to prevent any moisture formation inside the bag.
2. **Reagent B1 - Enzymatic tracer** : 1 vial of 0.8 ml.  
Proteic buffer solution containing anti-human IgM polyclonal antibody conjugated with HRP, 20 x concentrated. It contains proteic stabilizers, 0.2 mg/ml gentamicin sulphate and 0.3% Kathon GC as preservatives.
3. **Reagent B2 – Tracer diluent** : 1 vial of 16 ml.  
Proteic buffer solution for the preparation of the tracer. It contains 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, a detergent and preservatives (0,1% Kathon GC). 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 mixture of 1M hydrochloric and phosphoric acids.  
**Warning : The reagent is irritant. Handle with care.**
7. **Negative Control** : 1 vial of 2 ml.  
Ready-to-use prediluted human serum base, not reactive for anti-EBV-EA IgM antibodies.  
It contains proteic stabilizers, 0.1% sodium azide and 0.3% Kathon GC as preservatives.
8. **Positive Control** : 1 vial of 2 ml.  
Ready-to-use prediluted human serum base, reactive for anti-EBV-EA IgM antibodies.  
It contains proteic stabilizers, 0.1% sodium azide and 0.3% Kathon GC as preservatives.
9. **Reagent G - Sample diluent** : 2 vial 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.
10. **Reagent G1 – Neutralizing Reagent** : 1 vial of 10 ml.  
Buffered proteic solution for the neutralization of RF positive samples and IgG; it contains proteic stabilizers, 0.1% sodium azide and 0.3% Kathon GC as preservatives.
11. **Cardboard sealers** : 2 cardboard sealers to be used to cover the plate during the incubations.

## 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.  
When preparing chromogen/substrate we recommend the use of plastic disposable sterile containers.
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 EBV EA-IgM 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 when stored at 2-8°C 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, 200 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 -20°C. Avoid repeated freeze-thawing cycles.

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 hemolyzed samples can give uncorrect analytical results.

## REAGENTS PREPARATION

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

## 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 reagents, controls 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 the controls and the 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 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 "blinking" of the instrument is to be carried out in the blank reagent well (well A1).

## ASSAY SCHEME

1. **Predilute the sample 1:100 with the Sample Diluent (Ex.: 10 µl sample + 1000 µl diluent). Controls are ready-to-use; do not dilute! Mix carefully on vortex before dispensing.**
2. **Dispense 50 µl Neutralizing Reagent in all wells, except for the blanking A1 well and for controls. Follow this scheme :**

First incubation	REAGENTS	EBV-VCA peptides coated wells		
		A1 BLANK	CONTROLS	SAMPLE
First incubation	Controls	-	100 µl	-
	Sample	-	-	50 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	Reagent 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 for the negative control is lower than 0.250. Higher values indicate an uncorrect washing procedure. In such a case, check the efficiency of the washing device.
3. The OD 450 nm of the positive control is higher than 0.500. Lower values indicate kit or positive control decay. Before repeating the assay, check the expiry date of the kit

### CALCULATION OF RESULTS

Calculate the mean values of Negative Control (NC) and Positive Control (PC) and check for assay validity. Calculate the cut-off value through the following formula :

$$\text{cut-off} = \text{Mean NC} + 0.250$$

Calculate the mean OD 450 nm value of the samples.

Samples with OD 450 nm values higher than cut-off have to be considered reactive to anti EBV-EA IgM antibodies. Samples with OD 450 nm values lower than cut-off have to be considered not reactive.

**EXAMPLE OF CALCULATION**

Controls	O.D.
NC	0.050
PC	1.020
CO = NC + 0.250	0.300
Sample	0.650+

**CHARACTERISTICS OF THE ASSAY**

SENSITIVITY : >99%.  
 SPECIFICITY : >95% on plasma and sera.  
 REPRODUCIBILITY : CV between 2-9.5%.

**CLINICAL FEATURES IN EBV INFECTIONS**












Clinical trials made with the kits available for the EBV complex gave following analytical characteristics :

- \* Early acute primary infection phase:
  - presence of VCA IgM
  - absence of VCA IgG
  - possible presence of EBNA-1 IgM
  - absence of EBNA-1 IgG
- \* Late acute primary infection phase:
  - presence of VCA IgM
  - presence of VCA IgG at low avidity
  - presence of EBNA-1 IgM
  - presence of EBNA-1 IgG
- \* Convalescence or post-infective phase:
  - absence of VCA IgM
  - presence of VCA IgG at high avidity
  - absence of EBNA-1 IgM
  - presence of EBNA-1 IgG
- \* Re-activation phase (in immunosuppression or deficiency):
  - presence of VCA IgM with low titre
  - presence of VCA IgG at high avidity
  - presence of EBNA-1 IgM with low titre
  - presence of EBNA-1 IgG

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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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