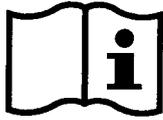


# Product information

Information about other products is available at: [www.demeditec.com](http://www.demeditec.com)



User's Manual

## Epstein-Barr Virus (EBNA-1) IgG ELISA



**DE4246**



**96 wells**



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## 1 INTRODUCTION

### 1.1 Intended Use

The **Epstein-Barr Virus (EBNA-1) IgG Enzyme Immunoassay Kit** provides materials for the **qualitative** and **semiquantitative** determination of IgG-class antibodies to Epstein-Barr virus nuclear antigen type 1 (EBV-EBNA-1) in human serum or plasma.

**This assay is intended for in vitro diagnostic use only.**

### 1.2 Summary and Explanation

Epstein-Barr Virus (EBV) is a member of the herpesvirus family (Gamma subgroup, DNA virus of 120-200 nm) and one of the most common human viruses. The virus occurs worldwide, and most people become infected with EBV sometime during their lives. Transmission of the virus is almost impossible to prevent since many healthy people can carry and spread the virus intermittently for life. Infants become susceptible to EBV as soon as maternal antibody protection disappears. Infection of children usually causes no symptoms. Infection during adolescence or young adulthood causes infectious mononucleosis 35% to 50% of the time. Infectious mononucleosis is almost never fatal. There are no known associations between active EBV infection and problems during pregnancy, such as miscarriages or birth defects. Although the symptoms of infectious mononucleosis usually resolve in 1 or 2 months, EBV remains dormant or latent in a few cells in the throat and blood for the rest of the person's life. Periodically, the virus can reactivate and is commonly found in the saliva of infected persons. This reactivation usually occurs without symptoms of illness. EBV also establishes a lifelong dormant infection in some cells of the body's immune system. A late event in a very few carriers of this virus is the emergence of Burkitt's lymphoma and nasopharyngeal carcinoma, but EBV is probably not the sole cause of these malignancies.

## 2 PRINCIPLE OF THE TEST

The **DEMEDITEC Epstein-Barr Virus (EBNA-1) IgG ELISA Kit** is a solid phase enzyme-linked immunosorbent assay (ELISA) Microtiter wells as a solid phase are coated with recombinant EBV nuclear antigen type 1. **Diluted patient** specimens and **ready-for-use controls** are pipetted into these wells. During incubation EBNA-1-specific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgG antibodies are dispensed into the wells. During a second incubation this anti-IgG conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of EBNA-1-specific IgG antibody in the patient specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

### 3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Avoid contact with Stop Solution containing 0.2 mol/L H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns.
5. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
6. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided
7. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
8. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
9. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
10. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
11. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
12. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
13. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
14. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
15. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
16. Do not use reagents beyond expiry date as shown on the kit labels.
17. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
18. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
20. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Demeditec.

## 4 KIT COMPONENTS

### 4.1 Contents of the Kit

1. **SORB MT Microtiterwells**, 12 x 8 (break apart) strips, 96 wells; Wells coated with recombinant EBV nuclear antigen type 1. (incl. 1 strip holder and 1 cover foil)
2. **SAM DIL Sample Diluent** \*, 1 vial, 100 mL, ready to use, colored yellow; pH  $7.2 \pm 0.2$ .
3. **CAL C Pos. Control** \*, 1 vial, 2.0 mL, ready to use; colored yellow, red cap.
4. **CAL A Neg. Control** \*, 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.
5. **CAL B Cut-off Control** \*, 1 vial, 2.0 mL, ready to use; colored yellow, black cap.
6. **ENZ CONJ Enzyme Conjugate** \*, 1 vial, 20 mL, ready to use, colored red, antibody to human IgG conjugated to horseradish peroxidase.
7. **SUB TMB Substrate Solution**, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
8. **STOP SOLN Stop Solution**, 1 vial, 14 mL, ready to use, contains 0.2 mol/L  $H_2SO_4$ , Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **WASH SOLN 20x Wash Solution** \*, 1 vial, 30 mL (20X concentrated for 600 mL), pH  $6.5 \pm 0.1$  see „Preparation of Reagents“.

\* contain non-mercury preservative

#### 4.1.1 Equipment and material required but not provided

- A microtiter plate calibrated reader (450/620 nm  $\pm 10$  nm)
- Calibrated variable precision micropipettes
- Incubator 37 °C
- Manual or automatic equipment for rinsing wells
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Timer
- Absorbent paper

### 4.2 Storage and stability of the Kit

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for two months if stored as described above.

### 4.3 Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

#### Wash Solution

Dilute Wash Solution **1+19** (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH value of  $7.2 \pm 0.2$ .

Consumption: ~ 5 mL per determination. Crystals in the solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use. The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

### 4.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

### 4.5 Damaged Test Kits

In case of any severe damage to the test kit or components, Demeditec has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

## 5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA-, heparin- or citrate\* plasma) can be used in this assay. (If \*citrate plasma is used, results could be little lower.) Do not use haemolytic, icteric or lipaemic specimens.

### 5.1 Specimen Collection

#### Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

#### Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

### 5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

### 5.3 Specimen Dilution

Prior to assaying dilute each patient specimen **1+100** with Sample Diluent; e.g. 10 µL of specimen + 1 mL of Sample Diluent. **Mix well and incubate for 15 minutes at room temperature, mix well again.**

**Please note:** Controls are ready for use and must not be diluted!

## 6 ASSAY PROCEDURE

### 6.1 General Remarks

- **It is very important to bring all reagents, samples and controls to room temperature before starting the test run!**
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- During 37°C incubation cover microtiter strips with foil to avoid evaporation.

## 6.2 Test Procedure

Prior to commencing the assay, dilute Wash Solution, **prepare patient samples as described in point 5.3** and establish carefully the **distribution and identification plan** supplied in the kit for all specimens and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,	
1 well	(e.g. B1)	for the Neg. Control,	
2 wells	(e.g. C1+D1)	for the Cut-off Control	and
1 well	(e.g. E1)	for the Pos. Control.	

It is left to the user to determine controls and patient samples in duplicate.

2. Dispense
  - 100 µL** of Neg. Control into well B1
  - 100 µL** of Cut-off Control into wells C1 and D1
  - 100 µL** of Pos. Control into well E1 and
  - 100 µL** of each diluted sample with new disposable tips into appropriate wells.

Leave well A1 for substrate blank!
3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37 °C**.
4. Briskly shake out the contents of the wells.  
Rinse the wells **5 times** with diluted Wash Solution (**300 µL per well**). Strike the wells sharply on absorbent paper to remove residual droplets.  
**Important note:**  
The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
5. Dispense **100 µL** Enzyme Conjugate into each well, **except A1**.
6. Incubate for **30 minutes at room temperature (20 °C to 25 °C)**.  
Do not expose to direct sun light!
7. Briskly shake out the contents of the wells.  
Rinse the wells **5 times** with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add **100 µL** of Substrate Solution into all wells.
9. Incubate for **exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark**.
10. Stop the enzymatic reaction by adding **100 µL** of Stop Solution to each well.  
Any blue color developed during the incubation turns into yellow.  
**Note:** Highly positive patient samples can cause dark precipitates of the chromogen!
11. Read the optical density at **450/620 nm** with a microtiter plate reader **within 30 minutes** after adding the Stop Solution.

## 6.3 Measurement

**Adjust** the ELISA microplate or microstrip reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

**Measure the absorbance** of all wells at **450 nm** and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable **calculate the mean absorbance values** of all duplicates.

## 7 RESULTS

### 7.1 Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

<b>Substrate blank in A1:</b>	Absorbance value <b>lower than 0.100</b>
<b>Neg. Control in B1:</b>	Absorbance value <b>lower than 0.200</b>
<b>Cut-off Control in C1/D1 :</b>	Absorbance value <b>between 0.350 – 0.850</b>
<b>Pos. Control in E1.</b>	Absorbance value <b>between 0.650 – 3.000</b>

### 7.2 Calculation

#### Mean absorbance value of Cut-off Control [CO]

Calculate the mean absorbance value of the two (2) Cut-off Control determinations (e.g. in C1/D1).

**Example:**  $(0.44 + 0.46) / 2 = 0.45 = \text{CO}$

### 7.3 Interpretation

**POSITIVE** Patient (mean) absorbance values more than 10 % above CO  
(Mean OD patient  $> 1.1 \times \text{CO}$ )

**GREY ZONE** Patient (mean) absorbance values from 10 % above to 10 % below CO  
repeat test 2 - 4 weeks later - with new patient samples  
( $0.9 \times \text{CO} \leq \text{Mean OD patient} \leq 1.1 \times \text{CO}$ )

Results in the second test again in the grey zone  $\Rightarrow$  **NEGATIVE**  
**NEGATIVE** Patient (mean) absorbance values more than 10 % below CO  
(Mean OD patient  $< 0.9 \times \text{CO}$ )

#### 7.3.1 Results in Units [U]

$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{CO}} = [\text{Units} = \text{U}]$

**Example:**  $\frac{1.580 \times 10}{0.45} = 35 \text{ U}$

#### Interpretation of Results

Cut-off value: 10 U  
Grey zone: 9 - 11 U  
Negative: < 9 U  
Positive: > 11 U

## 8 QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or DEMEDITEC directly.

## 9 ASSAY CHARACTERISTICS

### 9.1 Assay Dynamic Range

The range of the assay is between 0.62 - 60 U/mL.

### 9.2 Analytical Sensitivity

The analytical sensitivity of the ELISA was calculated by adding 2 standard deviations from the mean of 20 replicate analyses of the negative control and was found to be 0.62 U/mL ( $OD_{450} = 0.033$ ).

### 9.3 Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. (Detected by method comparison with Virion-Serion ELISA, with three lots of Demeditec ELISA. 92 samples, therefrom 37 negative samples are assayed). It is 100% for all three Demeditec production lots.

### 9.4 Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. (Detected by method comparison with Virion-Serion ELISA, with three lots of Demeditec ELISA. 92 samples, therefrom 55 positive samples are assayed). It is 100% for all three Demeditec production lots.

### 9.5 Method Comparison

The Demeditec EBV (EBNA-1) IgG ELISA was compared with the Virion-Serion EBV (EBNA-1) IgG ELISA. 92 serum samples are assayed.

	n= 92	Virion-Serion	
		pos.	neg.
Demeditec ELISA Lot 1	pos.	55	0
	Neg.	0	37

**Agreement: 100%**

### 9.6 Reproducibility

9.6.1 The intra-assay (within-run) precision of the Demeditec EBV-EBNA-1 IgG ELISA was determined by 20 x measurements of 12 serum samples covering the whole measuring range.

Sample	Mean $OD_{450}$	Intra-Assay CV (%)	n
1	0,21	5,26	20
2	0,31	4,78	20
3	0,21	4,13	20
4	0,92	2,36	20
5	0,82	4,36	20
6	0,65	3,78	20
7	1,40	2,49	20
8	1,40	2,37	20
9	1,11	1,58	20
10	1,55	4,34	20
11	1,62	2,51	20
12	1,52	3,74	20

9.6.2 The inter-assay variation of the Demeditec EBV-EBNA-1 IgG ELISA was determined with 3 samples with 2 production kits in 10 independent runs with 2 replicates per run.

Sample	Mean $OD_{450}$	Inter-Assay CV (%)	n
1	1,45	4,17	40
2	0,22	14,26	40
3	1,04	4,87	40

## 10 LIMITATIONS OF USE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. In immunocompromised patients and newborns serological data only have restricted value.

## 11 LEGAL ASPECTS

### 11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact Demeditec.

### 11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

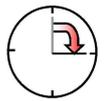
### 11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

## REFERENCES / LITERATURE

1. Lamy ME., Favart AM., Cornu A. et al., "Study of Epstein-Barr virus (EBV) antibodies: IgG and IgM anti-VCA, IgG anti EA and Ig anti-EBNA obtained with an original microtiter technique. Serological criteria of primary and recurrent EBV infections and follow-up of infectious mononucleosis. Seroepidemiology of EBV in Belgium based on 5178 sera from patients". *Acta Clin. Belg.*, 37 (5): 281-298, (1982)
2. Lennette E., „Epstein-Barr Virus“, in *Manual of Clinical Microbiology*, 4th ed. Washington D.C., Am. Soc. Microbiol. P 728-732, (1985)
3. Luka J., Chase PC., Pearson GR., "A sensitive enzyme-linked Immunosorbent assay (ELISA) against the major EBV-associated antigens. I-Correlation between ELISA and immunofluorescence titers using purified antigens", *J. Immunol. Metho.*, 67: 145-156, (1984)

**SHORT INSTRUCTIONS FOR USE**

	All reagents and specimens must be allowed to come to room temperature (18-25°C) before use. Prepare Wash Solution and <b>dilute Samples</b> .
	Leave well A1 for substrate Blank. Dispense 100 µL of Controls into appropriate wells.
	Dispense 100 µL of sample into selected wells. <b>(Please note special sample treatment, point 5.3!)</b>
	Cover wells with foil. Incubate for <b>60 minutes</b> at 37 °C.
	Briskly shake out the contents of the wells.
	Rinse the wells <b>5 times</b> with diluted Wash Solution (300 µL per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Dispense 100 µL of Enzyme-Conjugate into each well.
	Incubate for <b>30 minutes</b> at room temperature.
	Briskly shake out the contents of the wells.
	Rinse the wells <b>5 times</b> with diluted Wash Solution (300 µL per well).
	Strike the wells sharply on absorbent paper to remove residual droplets.
	Add 100 µL of Substrate Solution to each well.
	Incubate for <b>15 minutes</b> at room temperature.
	Stop the reaction by adding 100 µL of Stop Solution to each well.
	Determine the absorbance of each well at 450 nm.

**SYMBOLS USED WITH DEMEDITEC ASSAYS**

Symbol	English	Deutsch	Français	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<b>Distributed by</b>	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore