RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgG.

Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1.
Doubtful: if +/- 10% of the Cut-Off.
Negative: if the ratio is < 0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

Subtract the value of the blank from all the other readings. The OD values of Cut-Off control must be at least 0.2.
Positive control must have an OD at least 1.5 times that of Cut-Off.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

95 human sera were analyzed by this EBV Early IgG Elisa and an Elisa reference method. Out of 95 samples, 41 were positive for the presence of IgG antibodies to EBV Early by DIAsource Elisa and reference Elisa showed 41 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
DIA	41	54	0	0
Reference	41	54	0	0

2. Precision

2. Inter-assay Study			
No of Replicates 10	Serum 1	Serum 2	Serum 3
Mean (OD's)	0.912	0.554	0.036
SD	0.023	0.028	0.034
CV%	**2,5**	**5,1**	**9.6**

3. Intra-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean (OD's)	1.07	0.700	0.044
SD	0.016	0.013	0.002
CV%	**1.5**	**1.85**	**4.9**

3. Interference study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

REFERENCES

1. A. Andersson, V. Vetter et al. Avidities of IgG directed against viral capsid antigen or early antigen: useful markers for significant Epstein-Barr Virus serology. J. Med. Virology 43: 238 (1994).
2. J.Middeldorp and P. Herbrink: Epstein Barr Virus specific marker molecules for early diagnosis of infectious mononucleosis. J. Virol. Methods 21: 133 (1988).
3. C. Valent Sumaya: Serological testing for Epstein Barr Virus - developments in interpretation. J. Inf. Dis. 151: 984 (1985).
4. J. Luka, R.C. Chase and G. Pearson: 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.

Catalogue Nr : KAPREEG40 PI Nr : 1701262 Revision Nr : 101018/1

Epstein Barr Virus Early IgG Elisa

IVD CE

2°c 8°c

Catalog No. KAPREEG40

DIAsource ImmunoAssays SA	Phone : + 32 67 88 99 99
Rue de l'Industrie 8	Fax : + 32 67 88 99 90
1400 Nivelles	Email : tech.support@diasource.be
Belgium	Website : www.diasource.be

INTENDED USE

The DIAsource KAPREEG40 Epstein Barr Virus EBV Early IgG Elisa test system is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to the early antigen of EBV in human serum. This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

Epstein Barr Virus (EBV) is a herpes virus, which causes infectious mononucleosis (IM). It is also associated with Burkitt's lymphoma, nasopharyngeal carcinoma and lymphatic proliferative syndromes in immunodepressed patients. The virus is widespread throughout the world and 80-90% of the population is serum-positive.

The laboratory diagnosis of IM is traditionally performed by detecting heterophile antibodies which develop in the serum during the course of the infection and which agglutinate horse erythrocytes. However, these antibodies may not always be present in patients affected by IM, particularly if below 14 years of age; furthermore, they may also persist for over a year after the infection. The determination of heterophile antibodies alone may therefore lead to an erroneous diagnosis. It is therefore important to determine the presence of antibodies towards the viral antigens. The detection of antibodies directed to the "Viral Capsid Antigen" (VCA) and the nuclear antigen (EBNA) is particularly useful. During the course of IM, the IgM- and IgG-class antibodies to VCA very appear early and followed to eary antigen, while the IgG to EBNA develop later during the infection. The presence of IgM against VCA,early in the absence of IgG against EBNA therefore indicates that there is a current infection, while the presence of IgG against both VCA, Early and EBNA is indicative of a prior infection.

PRINCIPLE OF THE TEST

The KAPREEG40 EBV Early IgG kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with recombinant derived Epstein Barr Virus early antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-EBV Early IgG antibodies present.

REAGENTS

EBV Early Antigen-Coated Microtitration Strip		Quantity : 1 plate
Wash Concentrate	WASH SOLN CONC	Quantity : 1 bottle
Sample Diluent	DIL SPE	Quantity : 1 bottle
TMB-Substrate	CHROM TMB	Quantity : 1 bottle
Negative control	CONTROL L	Quantity : 1 vial
Cut off control	CONTROL CO	Quantity : 1 vial
Positive control	CONTROL H	Quantity : 1 vial
2nd Antibody Conjugate	Ab HRP	Quantity : 1 bottle
Stopping Solution	STOP SOLN	Quantity : 1 bottle

MATERIAL NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µL, 100 µL and 1 mL
- Semi-automatic pipette to deliver 100 µL
- Automatic microtitration plate washer
- Absorbent material for blotting the strips
- Incubator capable of maintaining a temperature of 37°C +/- 1 °C

Antigen-Coated Microtitration Strips
One stripholder containing 12x8 (96) microtitration wells coated with recombinant derived EBV early antigen. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate
One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% per weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent
One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

EBV Early IgG Controls
Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

2nd Antibody Conjugate
One bottle, 12 mL, containing anti-human IgG monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

TMB-Substrate
One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution
One bottle, 15 mL, containing 0.3 M H_2SO_4 in solution. Store at 2-8°C until expiration date.

PRECAUTIONS
For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain some reagents made with human ad animal source material (e.g. serum, plasma or bovine albumin) or used in conjunction with human and animal source materials. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HbsAg; the animal source material is also free from infection. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION:
Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.
For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING
Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at –20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY
A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affected the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS
Wash Solution

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

Microtitration Strips

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Assay Procedure
All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank.
4. Incubate for 45 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
6. Add 100 µL of Enzyme-Labeled 2nd Antibody into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µL of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

	Consult instructions for use		Manufacturer
	Storage temperature		Contains sufficient for n tests
	Use by	IVD	In vitro diagnostic medical device
LOT	Batch code	REF	Catalogue number

Revision date : 2010-10-18