Anti-EBV-CA ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2791-9601 G	Epstein-Barr virus capsid antigen (EBV-CA)	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the immunoglobulin class IgG against EBV-CA in serum or plasma for the diagnosis of infectious mononucleosis, Burkitt`s lymphoma and nasopharyngeal carcinoma.

Application: Primary and past EBV infections can be diagnosed reliably by the detection of antibodies of class IgG and/or IgM against EBV-CA, and of class IgG against EBNA-1. Exceptional serological constellations, such as primary infections without detectable anti-EBV-CA-IgM, persisting IgM, or a reactivation without formation of anti-EBNA-1 IgG (secondary anti-EBNA-1 IgG loss) can be clearly diagnosed by avidity determination of anti-EBV-CA IgG antibodies or the detection of further antibodies, e.g. against the late phase marker p22.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with EBV-CA. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example, for 1 microplate strip: 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light ✳. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl of sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantitative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash the reagent wells 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.
Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to 25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to 25°C; protect from direct sunlight).

Stopping:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7 to 10 is an example for the **quantitative analysis** of 24 patient sera (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

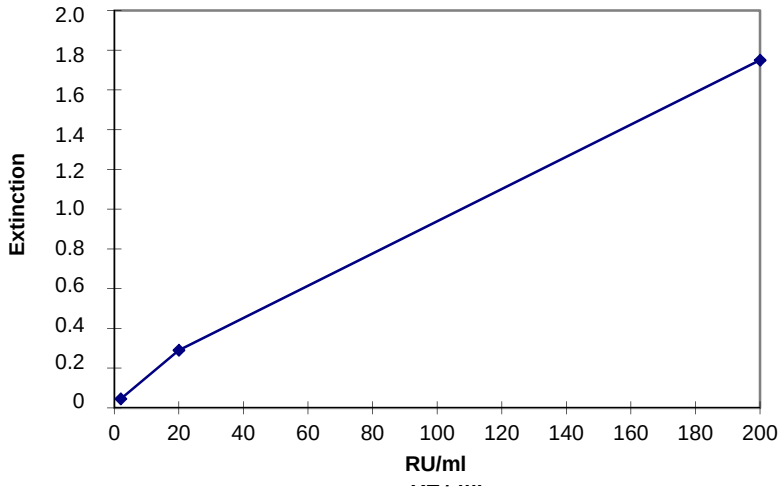
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator 2}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8: **negative**
Ratio ≥0.8 to <1.1: **borderline**
Ratio ≥1.1: **positive**

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as “>200 RU/ml”. It is recommended that the sample be retested at a dilution of e. g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	**negative**
≥16 to <22 RU/ml:	**borderline**
≥22 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Interpretation of serological constellations in EBV diagnostics:

		EBV-CA IgG	gp125 and/or p19 IgG	EBV-CA IgM	gp125 and/or p19 IgM	EBV-EA IgG	EBNA	IgG-avidity
No infection with EBV		-	-	-	-	-	-	-
Infectious mononucleosis	Early phase	+/++	+/++	+	+	++	-	low
	Late phase	+/++	+/++	+/-	+/-	+/++	+/-	high
	Convalescence	+/++	+/++	-	-	+/-	+	high
	Infection a long time ago	+	+	-	-	+/-	+	high
	Reactivated	+	+	-	-	+/(-)	+	high
	Chronic	+	+	-	-	+/++	-	high
Special serological constellations	Early phase without detectable anti-CA-IgM or retarded formation of anti-IgM	+	+	-	-	++	-	low
	Past infection with persisting anti-VCA IgM	+/++	+/++	+	+	+/-	+	high
	Recent infection with IgM reactivation	+/++	+/++	+	+	+/-	+	high
	Early phase without detectable anti-EA IgG	+/++	+/++	+	+	-	-	low
	Past infection with anti-EBNA-1 loss	+/++	+/++	-	-	+/-	-	high
	Past infection with retarded or absent anti-EBNA-1 conversion	+/++	+/++	-	-	+/-	-	high
Burkitt`s lymphoma		++	++	-	-	++	+	high
Nasopharyngeal carcinoma (EBV-CA IgA ++; EBV-EA IgA +/++)		++	++	+/-	+/-	++	+	high
Reactivated infection		Determination of the viral load is indicated for characterisation of reactivated EBV infections in immunosuppressed patients or persons with a weakened immune system. In these cases the serological results are of limited relevance compared to the determination of the viral load.						

Test characteristics

Calibration: As no international reference serum exists for antibodies against EBV-CA, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with the purified Epstein-Barr virus capsid antigens. The antigen source is provided by inactivated cell lysates of human B cells infected with the “P3HR1” strain of Epstein-Barr viruses.

Linearity: The linearity of the Anti-EBV-CA ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-EBV-CA ELISA (IgG) is linear at least in the tested concentration range (4 RU/ml to 141 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-EBV-CA ELISA (IgG) is 0.9 RU/ml.

Cross reactivity: The quality of the antigen and the source of antigen (P3HR1-Cells EBV infected) used ensures a high specificity of the ELISA. No cross reactivities with Herpes viruses were determined. The test is anti-EBV specific.

Antibodies against	n	Anti-EBV-CA ELISA (IgG) positive
Adenovirus	10	0%
Chlamydia pneumoniae	5	0%
CMV	3	0%
Influenza virus A	4	0%
Influenza virus B	9	0%
Measles virus	9	0%
Mumps virus	9	0%
Mycoplasma pneumoniae	3	0%
Parainfluenza virus Pool	10	0%
RSV	8	0%
Rubella virus	10	0%
VZV	5	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	47	7.4
2	90	5.8
3	93	4.2

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	47	8.2
2	90	3.2
3	93	5.4

Sensitivity and specificity: 175 clinically pre-characterised patient samples (INSTAND and Labquality) were investigated with the EUROIMMUN Anti-EBV-CA ELISA (IgG). The sensitivity amounted to 100%, with a specificity of 100%. Borderline results were not included in the calculation.

n = 175		**INSTAND/Labquality**		
		positiv	borderline	negativ
EUROIMMUN Anti-EBV-CA ELISA (IgG)	positive	145	0	0
	borderline	3	1	0
	negative	0	0	26

Reference range: The levels of the anti-EBV-CA antibodies (IgG) were analysed with this EUROIMMUN ELISA in a collective of 500 healthy blood donors. With a cut-off of 20 RU/ml, 93.4% of the blood donors were anti-EBV-CA positive (IgG) which reflects the known percentage of infections in adults.

Clinical significance

Epstein-Barr virus (EBV) is one of the most widely distributed human-pathogenic herpes viruses. The virus is transmitted by smear infection, but also by blood transfusions or organ transplants. EBV is the causative agent of infectious mononucleosis (Pfeiffer's disease), a febrile disease usually accompanied by pharyngitis and lymphadenopathy, frequently by hepatosplenomegaly and more rarely by an exanthema. When first infection occurs in childhood the disease often proceeds without symptoms. In industrial countries mainly adolescents or young adults become infected, often leading to manifest disease. EBV infections are also found in connection with the pathogenesis of malignant lymphoma (endemic form of Burkitt's lymphoma in Africa) and nasopharyngeal carcinoma (NPC, especially widespread in South-East Asia). NPC is the third most frequent malignant tumour in southern China. Since 2011 the evidence has been increasing that EBV infection is associated with a high risk of multiple sclerosis (MS) or an aggravation of MS.

The main goal of EBV diagnostics in people with a healthy immune system is to differentiate between an acute and a past infection. Various serological methods are used for this. The immune system of healthy persons can quickly suppress a reactivation of the virus. However, in immunosuppressed patients (e. g. those on immunosuppressive therapy after organ transplantation or with an HIV infection) EBV can spread uncontrollably and cause severe lymphoproliferative diseases. In such cases it is diagnostically very important to also determine the viral load, for which PCR (polymerase chain reaction) is normally used.

Infectious mononucleosis must be differentiated from cytomegalic inclusion body disease and toxoplasmosis and, in the case of atypical progress, also from HIV infection or other infections.

In pregnancy, EBV can cause infection of the placenta, leading to damage to the foetal heart, eyes and liver. In children, accompanying infections of the kidney have been observed with symptoms from microscopic haematuria to acute kidney failure.

The immune response to an EBV infection is characterised by the formation of antibodies against EBV capsid, EBV nuclear and EBV early antigens successively.

In the early phase of the disease IgM and IgG antibodies against the viral capsid antigen (CA) can be detected. A positive Anti-EBV-CA (IgM) result is the classical marker of acute infection. IgG antibodies against early antigen are produced later in the acute phase and decrease after three to six months to a non-detectable level. Anti-CA IgG antibody levels persist lifelong. Around six to eight weeks after infection, antibodies against EBNA are produced. The presence of anti-EBNA antibodies indicates a past infection.

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