Version 21-04/24

User's Manual

Human Epstein-Barr virus early antigen diffuse (EBV-EA-D) IgG ELISA Kit

REF **DEIA329**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The ELISA test kit provides semiquantitative or quantitative in vitro determination of human antibodies of the immunoglobulin class IgG against Epstein-Barr virus early antigen diffuse (EBV-EA-D) in serum or plasma to support the diagnosis of infectious mononucleosis.

General Description

Since early EBV antigens (early antigen, EA) are expressed in the lytic replication phase, the detection of specific antibodies using the Anti-EBV-EA-D IgG ELISA Kit can contribute to supporting the diagnosis of active EBV infections. Results should always be interpreted within the context of clinical symptoms and with respect to further laboratory diagnostic analyses.

Principles of Testing

The test kit contains microtiter strips each with 8 break-off reagent wells coated with EBV-EA-D. 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), which is capable of promoting a colour reaction.

Reagents And Materials Provided

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
2. Calibrator 1, 200 RU/ml (IgG, human), ready for use, dark red, 1 x 2.0 ml
3. Calibrator 2, 20 RU/ml (IgG, human), ready for use, red 1 x 2.0 ml
4. Calibrator 3, 2 RU/ml (IgG, human), ready for use, light red, 1 x 2.0 ml
5. Positive control, (IgG, human), ready for use, blue 1 x 2.0 ml
6. Negative control, (IgG, human), ready for use green 1 x 2.0 ml
7. Enzyme conjugate, peroxidase-labelled anti-human IgG (rabbit), ready for use, green, 1 x 12 ml
8. Sample buffer, ready for use, light blue, 1 x 100 ml
9. Wash buffer, 10x concentrate, colourless, 1 x 100 ml
10. Chromogen/substrate solution, TMB/H_2O_2, ready for use, colourless, 1 x 12 ml
11. Stop solution, 0.5 M sulphuric acid, ready for use, colourless, 1 x 12 ml
12. Test instruction --- 1 booklet
13. Quality control certificate --- 1 protocol

Storage

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

Specimen Collection And Preparation

Samples: 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 in 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: Calibrators and controls are prediluted and ready for use, do not dilute them.

Plate Preparation

	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 samples (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. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises 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.

Reagent Preparation

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 ready-for-use diluted 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.

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

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, antiHIV-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 sodium azide in a non-declarable concentration. Avoid skin contact.

Assay Procedure

For semiquantative 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.

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 readings. 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 readings. 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.

Calculation

Semiquantitative:

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

Extinction of the control or patient sample/Extinction of calibrator 2 = Ratio

We recommend interpreting results as follows:

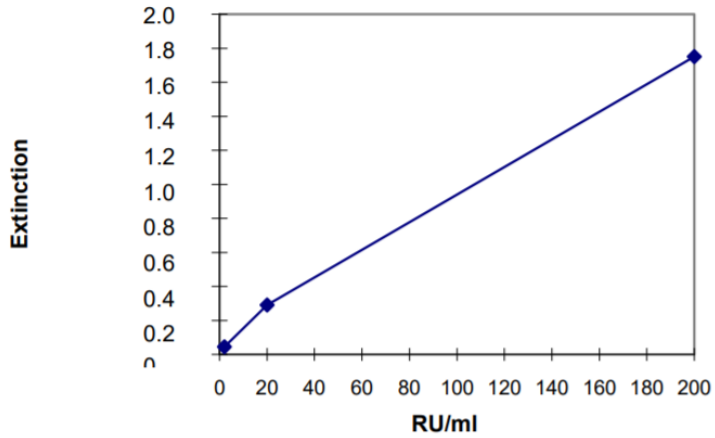
Ratio <0.8: negative

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 readings 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 extinction of calibrator 1 (corresponding to 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 factor 4.

The upper limit of the normal range of non-infected persons (cut-off value) recommended by Creative Diagnostics is 20 relative units (RU)/ml. We recommend 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, Creative Diagnostics 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 IgG titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after at least 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.

Reference Values

The levels of the anti-EBV-EA-D antibodies (IgG) were analysed with this ELISA in a panel of 297 healthy blood donors. With a Cut-off of 20 RU/ml, 5% of the blood donors were anti-EBV-EA-D positive (IgG).

Performance Characteristics

Calibration:

As no international reference serum exists for antibodies against EBV-EA-D, the calibration is performed in relative units (RU). For every group of tests performed, the extinction readings 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 is 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 incubation steps, the greater will be the extinction. 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 largelycompensated in the calculation of the result.

Antigen:

The microplate wells were coated with the recombinant Epstein-Barr virus early antigen diffuse. The protein was expressed in E. coli and the molecular weight is 45 kDa.

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 EBV- EA-D IgG ELISA kit is 0.8 RU/ml.

Sensitivity

n = 35		INSTAND		
		positive	borderline	negative
Anti-EBV-EA-D ELISA (IgG)	positive	3	0	0
	borderline	0	0	0
	negative	0	2	30

Specificity

EBV

Linearity

The linearity of the EBV-EA-D IgG ELISA Kit was determined by assaying at least 4 serial dilutions of different patient samples. The kit is linear at least in the tested concentration range (2 RU/ml to 158 RU/ml).

Reproducibility

The reproducibility of the test was investigated by determining the intra- and interassay 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.

Inter-assay variation, n = 4 x 6		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	33	4.5
2	107	7.0
3	146	5.2

Intra-assay variation, n = 20		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	32	4.0
2	102	4.1
3	142	3.1

Interferences

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.

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