Epstein Barr Virus
EBNA-1 IgM ELISA Kit

Catalog Number KA1449
96 assays
Version: 03
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Introduction

Intended Use

The Epstein Barr Virus EBNA-1 IgM ELISA Kit is for detection of IgM antibodies to Epstein-Barr virus (EBV) nuclear antigen-1 (EBNA-1) in serum or plasma samples.

Principle of the Assay

The Epstein Barr Virus EBNA-1 IgM ELISA Kit is a solid-phase immunoanalytical test. The polystyrene strips are coated with antigen that bears immunodominant epitopes of EBNA-1. The anti-EBNA-1 EBV antibodies, if present in the tested sera, bind to the immobilized antigens and the antibodies being in complexes with antigen are later on recognized by animal anti-human IgM antibodies labelled with horseradish peroxidase. The labelled antibodies are revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in colour, if present, may be attributed to the reaction background.
Flow Chart

Step 1. Prepare reagents and samples.

↓

Step 2. Dispense 100 μL/well of Dilution buffer, Controls and samples.

↓

Incubate 30 minutes at 37°C

↓

Wash 4 times (250 μL/well), aspirate

↓

Step 3. Dispense 100 μL/well of Px-conjugate r.t.u.

↓

Incubate 30 minutes at 37°C

↓

Wash 4 times (250 μL/well), aspirate

↓

Step 4. Dispense 100 μL/well of TMB substrate

↓

Incubate 15 minutes in dark at room temperature

↓

Step 5. Dispense 100 μL/well of Stop solution

↓

Step 6. Read absorbance at 450/620-690 nm within 20 min
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA break-away strips coated with specific antigens.</td>
<td>1 microplate</td>
</tr>
<tr>
<td>Calibrator, r.t.u.</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>Positive control serum, r.t.u.</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>Negative control serum, r.t.u.</td>
<td>1.3 mL</td>
</tr>
<tr>
<td>Anti-human IgM antibodies labeled with horseradish peroxidase (Px-conjugate) r.t.u.</td>
<td>13 mL</td>
</tr>
<tr>
<td>Wash buffer, 10x concentrated</td>
<td>55 mL</td>
</tr>
<tr>
<td>Dilution buffer, r.t.u.</td>
<td>60 mL</td>
</tr>
<tr>
<td>Chromogenic substrate (TMB substrate), r.t.u.</td>
<td>13 mL</td>
</tr>
<tr>
<td>Stop solution, r.t.u.</td>
<td>13 mL</td>
</tr>
</tbody>
</table>

r.t.u.: ready to use

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Storage Instruction

✓ The ELISA kit should be used within three months after opening.
✓ Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Store unused strips in the sealable pouch and keep the desiccant inside.
✓ Expiration date is indicated on the ELISA kit label and on all reagent labels.
✓ If you find damage at any part of the kit, please inform the manufacturer immediately.

Materials Required but Not Supplied

✓ Distilled or deionized water for dilution of the Wash buffer concentrate.
✓ Appropriate equipment for pipetting, liquid dispensing and washing.
✓ Thermostat (set at 37°C) for ELISA plate incubation.
✓ Spectrophotometer/colorimeter/microplate reader — wavelength 450 nm (and 620-690 nm reference filter-recommended, not required).

Precautions for Use

✓ Safety Precautions
  • Only for research use.
  • All ingredients of the kit are intended for laboratory use only.
• Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However, they should be regarded as contagious and handled and disposed of according to the appropriate regulations.

• Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

• Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

• The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, chloramine,….) in concentrations recommended by the producer.

• Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

✓ Handling Precautions

• Manufacturer guarantees performance of the entire ELISA kit.

• Follow the assay procedure indicated in the Instruction manual.

• Calibrator and control sera contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1)).

• Avoid microbial contamination of serum samples and kit reagents.

• Avoid cross-contamination of reagents.

• Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.

• Follow the assay procedure indicated in the Instruction manual.

• Variations in test results are usually due to:
  * Insufficient mixing of reagents and samples
  * Inaccurate pipetting and inadequate incubation times
  * Poor washing technique or spilling the rim of well with sample or Px-conjugate
  * Use of identical pipette tip for different solutions.
Assay Protocol

Reagent Preparation

1. Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.
2. Vortex samples and Control sera in order to ensure homogeneity and mix all solutions well prior use.
3. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with appropriate volume of distilled or deionized water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one month if stored at room temperature.
4. Do not dilute the Control sera, Calibrator, Px-conjugate, TMB substrate and Stop solution, they are ready to use.

Sample Preparation

1. Dilute serum samples 1:101 in Dilution buffer (e.g. 5 μL of serum sample + 500 μL of Dilution buffer).
2. Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.
3. Do not store diluted samples. Always prepare fresh.

Assay Procedure

Manufacturer will not be held responsible for results if manual is not followed exactly.

1. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. Withdraw an adequate number of strips and put the unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
2. Pipette Controls and samples according to the Plate Layout. Start with filling the first well with 100 μL of Dilution buffer to estimate the reaction background. Fill next two wells with 100 μL of Calibrator, next one well with Positive control serum and the following one well with Negative control serum. Fill the remaining wells with 100 μL of serum samples (S1, S2, S3,...). It is sufficient to apply samples as singlets, however, if you wish to minimize laboratory error apply the Calibrator in triplet, and Negative Control and samples in doublets. Incubate 30 minutes (±2 min) at 37°C.
3. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 μL/well of Wash buffer. Avoid cross-contamination between wells! If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.
4. Add 100 μL of diluted Px-conjugate r.t.u. into each well. Incubate 30 minutes (±2 min) at 37°C.
5. Aspirate and wash four times with 250 μL/well of Wash buffer (see point 3 of this paragraph).

6. Dispense 100 μL of TMB substrate into each well. Incubate 15 minutes (+/-30 seconds) at room temperature. The time measurement must be started at the beginning of TMB dispensing. Keep the strips in the dark during the incubation with TMB substrate.

7. Stop the reaction by adding 100 μL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate few times to ensure complete mixing of the reagents.

8. Measure the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use a reference reading at 620 - 690 nm.
Data Analysis

Calculation of Results

✓ Processing of results
Begin the processing with subtraction of the absorbance of the Dilution buffer well (background absorbance) from the absorbances in all other wells. If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

✓ Processing of results for the Qualitative interpretation
1. Compute the mean absorbance of the two wells with Calibrator. (If the Calibrator was applied in three parallels and one absorbance is different from the mean in more than 20%, then exclude the deviating well from the calculation and compute a new absorbance mean with using the other two wells)
2. Compute the cut-off value by multiplying the mean with absorbance of Calibrator by correction factor. The correction factor values determined for the particular lot of the kit is stated in the Quality control certificate.
3. Samples with absorbances lower than 90% of the cut-off value are considered negative and samples with absorbances higher than 110% of the cut-off value are considered positive. The samples with absorbance in the range of 90-110% of cut-off value are equivocal (see note in "Processing of using Positivity index (semiquantitative interpretation)")

✓ Processing of using Positivity index (semiquantitative interpretation)
Determine Positivity Index for each serum sample and cerebrospinal fluid samples as follows:
1. Compute the cut-off value (see the previous paragraph)
2. Compute the Positivity Index according to the following formula:

\[
\text{sample Positivity Index} = \frac{\text{sample absorbance}}{\text{cut-off value}}
\]

3. Express a serum reactivity according to Table 1

<table>
<thead>
<tr>
<th>Positive Index</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.90</td>
<td>negative</td>
</tr>
<tr>
<td>0.90 - 1.10</td>
<td>+/-</td>
</tr>
<tr>
<td>&gt; 1.10</td>
<td>Positive*</td>
</tr>
</tbody>
</table>

* on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample.
Example of calculation:

- Calibrator absorbance = 1.014; 1.099; 1.123
- Mean absorbance of Calibrator = 1.079
- Correction factor of Calibrator = 0.16
- Cut-off value = 1.079 x 0.16 = 0.173
- Sample absorbance = 0.550
- Sample Positivity Index = 0.550/0.173 = 3.18

Note! An equivocal sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the person, usually withdraw 1-2 weeks later.

Performance Characteristics

- Validity of the test
  The test is valid if:
  - The background absorbance (the absorbance of the Dilution buffer) is less than 0.150.
  - The mean absorbance of standards (control sera), and the ratio between the absorbance values of Positive Control serum/Calibrator are in the ranges stated in the Quality control certificate for the lot of this kit.

- Precision of the test
  The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different absorbance values.
  Example of absorbance (A) range of positive serum samples was (n=12): 0.496 – 2.009.
  - Intraassay variability
    The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot as absorbance of minimum 12 parallel wells for the particular microtitrate plate.
    Example:
    (n = number of parallels at the same microtitration plate, CV - variation coefficient)

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>±σ</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.876</td>
<td>0.038</td>
<td>4.3%</td>
</tr>
<tr>
<td>16</td>
<td>1.759</td>
<td>0.080</td>
<td>4.6%</td>
</tr>
</tbody>
</table>

- Interassay variability
  The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of absorbance of the same serum sample in several consecutive tests.
  (n = number of an independent examination of the same serum sample)
Spiking recovery test
The percentage of recovery was between 80-120%.

Sensitivity and specificity
Sensitivity of the test is 95.5%. Evaluation was performed on serum samples which were expected to be positive for anti-EBNA-1 EBV IgM antibodies (persons in acute phase infectious mononucleosis).
Specificity of the test is 95.5%. Specificity was determined on serum samples from healthy EBV negative blood donors expected to be IgM anti-EBNA1 – negative.

Interference
Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 100 mg/mL of triglycerides.

<table>
<thead>
<tr>
<th>n</th>
<th>A</th>
<th>±σ</th>
<th>min-max</th>
<th>CV</th>
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</thead>
<tbody>
<tr>
<td>16</td>
<td>0.065</td>
<td>0.008</td>
<td>0.050-0.076</td>
<td>12.1%</td>
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<tr>
<td>16</td>
<td>1.983</td>
<td>0.112</td>
<td>1.753-2.139</td>
<td>5.7%</td>
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<tr>
<td>16</td>
<td>1.098</td>
<td>0.093</td>
<td>0.954-1.230</td>
<td>8.5%</td>
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Resources

References

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<tr>
<td>A</td>
<td>Dilution Buffer</td>
<td>Calibrator</td>
<td>Calibrator</td>
<td>Positive control</td>
<td>Negative control</td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 3</td>
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