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User's Manual

Human BZLF1/EBV Zebra Gene ELISA kit

REF **DEIA-BJ732**

Σ **96T**

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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.

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PRODUCT INFORMATION

Intended Use

The Human BZLF1/EBV Zebra gene ELISA kit provides materials for the qualitative and semiquantitative determination of to EBV BZLF1 antigen in human serum, plasma, tissue homogenate and other body fluid.

This assay is intended for research use only.

Principles of Testing

This EBV-BZLF1 ELISA Kit is a solid phase enzyme-inked immunosorbent assay (ELISA).

Microtiter wells as a solid phase are coated with antibody against Epstein-Barr viral antigen BZLF1. Pretreated specimens and ready-for-use controls are pipetted into these wells. During incubation Epstein-Barr viral Zebra Gene BZLF1 antigen of postive specimens and controls are bound to the immobilized antbodies. After washing step to remove unbound sample and control material horseradish peroxdase conjugated anti-BZLF1 anibodies are dispensed into the wells. During a second incubation this HRP-antibody conjugate binds specifically to BZLF1 antigen 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 resutls) are detected by incubation with TMB substrate and development of a blue color. The blue color turns yellow after stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of Epstain-Barr viral antigen BZLF1. In the specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader.

Reagents And Materials Provided

1. Microtiterwells, 12 × 8 (break apart) strips, 96 wells; Wells coated with Epstein-Barr viral BZLF1 antibody. (incl. 1 strip holder and 1 cover foil)
2. Sample Dilute: 1 vial, 100 mL, ready to use, colored yellow, pH 7.2 ± 0.2.
3. Balance Buffer: 1 vial, 6.5 mL, ready to use, colored yellow.
4. Pos.Control: 1 vial, 1.0 mL, ready to use; colored yellow, red cap.
5. Neg.Control: 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.
6. Cut-off Control: 1 vial, 2.0 mL, ready to use; colored yellow, black cap.
7. Enzyme Conjugate: 1 vial, 20 mL, ready to use, colored red, antibody to BZLF1 conjugated to horseradish peroxidase.
8. Substrate Solution: 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
9. Stop Solution: 1 vial, 14 mL, ready to use, contains 0.2 mol/H_2SO_4. Avoid contact with the stop solution. It may cause skin irritations and bums.
10. Wash Solution: 1 vial, 30 mL (20× concentrated for 600 mL). pH 6.5 ± 0.1 see "Reagent Preparation" contain non-mercury preservative.

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450/620 nm ± 10 nm)

2. Calibrated variable precision micropipettes
3. Manual or automatic equipment for rinsing wells
4. Deionized or (freshly) distilled water
5. Timer
6. Absorbent paper
7. Incubator 37°C
8. Vortex tube mixer

Storage

When stored at 2°C to 8°C unopened reagents will retain reacivity 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.

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 hemolytic, icteric or lipemic specimens.

1. **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. Samples containing anticoagulant may require increased dotting time.

2. **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.

3. **Tissue homogenates**

   The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02 mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS with a glass homogenizer on ice. The resulting suspension was subjected to µLtrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500 ×g (or 5000 rpm). Remove the supernate and assay immediately or aliquot and store samples at -20°C-80°C.

4. **Cell lysates**

Cells should be lysed according to the following directions.

   1. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.

2. Wash cells three times in PBS.

3. Cells were resuspended in PBS and subjected to µLtrasonication for 3 times. Alternatively, freeze cells at -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.

4. Centrifuge at 1000×g (or 3000 rpm) for 15 minutes at 2-8°C to remove cellular debris.

5. Assay immediately or store samples at -20°C or -80°C.

5. **Cell culture supernatants and other body fluids**

Centrifuge cell culture media at 1000 × g (or 3000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20°C or -80°C.

NOTE:

1. Samples should be aliquoted and could be stored at 5 days at 2°C to 8°C, -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles.

2. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

4. Samples containing a visible precipitate must be clarified prior to use in the assay. Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.

5. Do not use heat-treated specimens.

6. **Specimen Dilution**

Prior to assaying each specimen is first to be diluted with Sample Diluent. To reduce the sample signal background these prediluted samples then have to be incubated with Balance Buffer.

1. Dilute each specimen 1 + 50 with Sample Diluent, e.g. 10 µL of specimen + 0.5 mL of Sample Diluent. Mix well.

2. Mix well the Balance Buffer before use.

3. Dilute this prediluted sample 1 + 1 with Balance Buffer, e.g. 60 µL prediluted sample + 60 µL Balance Buffer. Mix well.

4. Let stand at room temperature for at least 15 minutes, up to a maximum of 2 hours and mix well again.

5. Take 100 µL of these pretreated samples for the ELISA.

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

Reagent Preparation

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

Washing Buffer (20× conc.)

Dilute Washing Buffer 1 + 19; e.g. 10 mL Washing Buffer + 190 mL distilled water. This diluted wash solution has a pH value of 7.2 ± 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.

Assay Procedure

General Remarks

1. It is very important to bring all reagents, samples and controls to room temperature before starting the test run!
2. Once the test has been started, all steps should be completed without interruption
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. 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 interuption.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
7. To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
8. During 37°C incubation cover microtiter strips with foil to avoid evaporation.

Test Assay

Prior to commencing the assay, dilute Wash Solution, prepare samples as described in Measurement and establish carefully the distribution and Identification plan supplied in the kit for all specimens and controls.

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

rease 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

1 well and (e.g. E1) for the Pos. Control

It is left to the user to determine controls and 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

   100 µL of each pretreated 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 residue 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 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.

Measurement

Adlust 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 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.

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 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 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 CD directly.

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: (0.49 + 0.51)/2 = 0.50 = CO

Interpretation Of Results

1. **Validation of the Test Run**

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

   Substrate blank in A1: Absorbance value lower than 0.100

   Neg. Control in B1: Absorbance value lower than 0.200

   cut-off Control in C1/D1: Absorbance value between 0.360 - 0.850

   Pos. Control in E1: Absorbance value between 0.650 - 3.000

2. **Interpretation**

   POSITIVE: Sample (mean) absorbance values more than 10 % above CO

   (Mean OD sample > 1.1 × CO)

   GREY ZONE: Sample (mean) absorbance values from 10 % above to 10 % below CO, repeat test 2-4 weeks later-with new samples.

   (0.9 × CO ≤ Mean OD sample ≤ 1.1 × CO)

   Results in the second test again in the grey zone → NEGATIVE

   NEGATIVE: Sample (mean) absorbance values more than 10 % below CO

   (Mean OD sample < 0.9 × CO)

3. Results in CD Units [DU]

   Sample (mean) absorbance value × 10 / CO = CD Units = [DU]

   Example: 1.580 × 10 / 0.50 = 32 DU

   Interpretation of Results

   Cut-off value: 10 DU

   Grey zone: 9 - 11 DU

   Negative: < 9 DU

   Positive: > 11 DU

Detection Range

The range of the assay is between 0.37 - 60 DU/mL.

Specificity

No cross-reactivity was found for HSV 1+2, HSV-1, HSV-2, CMV antigens

Precautions

1. All reagents of this kit are intended for professional use only.
2. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
4. Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
5. Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
6. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
7. During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

   • In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
8. Personal precautions, protective equipment and emergency procedures:

   • Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

   • Exposure controls/personal protection: Wear protective gloves of nitril rubber or natural latex.

   • Wear protective glasses. Used according to intended use no dangerous reactions known.
9. Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
10. For disposal of laboratory waste the national or regional legislation has to be observed.

Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.