BOSTER

antibody and ELISA experts

Human Brucella IgM ELISA Kit (Direct EIA)

Catalog number: EK7022

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Human Brucella IgM ELISA Kit (Direct EIA)

Catalog Number: EK7022

Size: 96 wells/kit

Sample Type: Serum and Plasma

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

Diluted patient serum is added to wells coated with purified antigens. If antibody against target antigen is present, the antibody will bind to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The enzyme catalyzes the substrate yielding a blue color (Amax = 370nm and 652nm) that changes to yellow (Amax = 450nm) upon addition of a sulfuric or phosphoric acid stop solution. The intensity of the color developed is proportional to the amount of IgG specific antibody in the sample.

Kit Components

Description	Quantity
Microwell coated with Brucella antigen	12x8x1
Sample Diluent: 1 bottle (ready to use)	22 ml
Calibrator: 1 Vial (ready to use)	1ml
Positive Control: 1 vial (ready to use)	1ml
Negative Control: 1 vial (ready to use)	1ml
Enzyme conjugate: 1 bottle (ready to use)	12ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
Wash concentrate 20X: 1 bottle	25ml

Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

WARNINGS AND PRECAUTIONS

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1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Typically, specimens may be refrigerated at 2-8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10ul of the sample to 200ul of sample diluent. Mix well.
3. Dispense 100ul of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100ul sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300ul of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100ul of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300ul of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100ul of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100ul of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).

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3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

LIMITATION OF THE TEST

1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and shows no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. Lipemic or hemolyzed samples may cause erroneous results.

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