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Brucella IgM ELISA Kit

(Catalog # E4710-100; 100 assay; Storage at 4ºC)

I. Introduction:

Brucella is gram negative coccobacilli capable of infecting a wide range of animal and man. Of the three species causing human infection, *B. melitensis* is the most pathogenic followed by *B. suis and B. abortus*. Brucellosis is transmitted through contaminated and untreated milk and milk products and by direct contact with infected animals (cattle, sheep, goats, pigs, camels, buffaloes, and, very recently, seals), animal carcasses, and abortion materials. BioVision's ELISA kit is for the detection of Brucella IgG antibody in human serum. Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds 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 plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

II. Applications:

This ELISA kit is used for the detection of Brucella IgM in human serum samples.

III. Sample Type:

- Serum

IV. Kit Contents:

Components	E4709-100	Part Number
Microwells coated with Brucella abortus antigen	8-well x 12 strips	E4710-100-1
Sample Diluent	22 ml	E4710-100-2
Calibrator	1 ml	E4710-100-3
Positive Control	1 ml	E4710-100-4
Negative Control	1 ml	E4710-100-5
Enzyme conjugate	12 ml	E4710-100-6
TMB Substrate	12 ml	E4710-100-7
Stop Solution	12 ml	E4710-100-8
Wash Buffer (20X)	25 ml	E4710-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Distilled or deionized water

VI. Storage Conditions and Reagent Preparation:

- The entire kit may be stored at 4°C.
- Viscous samples should always be diluted in phosphate buffered saline or distilled water prior to pippetting
- Prepare 1X Wash buffer by adding 475 ml of distilled or deionized water. Store at room temperature (20-25 °C).
- Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- **Sample preparation:**
  Collect serum from blood samples.
  Samples may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing. Lipemic or hemolyzed samples may cause erroneous results.
  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.
  Samples with high RF and high autoimmune antibodies, can cause interferences in the assay

VII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

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 10 µl f the sample to 200 µl f sample diluent and mix well.
3. Dispense 100 µl of diluted **serum, calibrator and controls** into the appropriate wells. For blank, add 100 µl sample diluent. Tap the plate to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.

4. Aspirate liquid from all wells. Wash wells three times with 300 µl of **1X wash buffer**. Blot on absorbance paper or paper towel.
5. Dispense 100 µl 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 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of **TMB substrate** and incubate for 10 minutes at room temperature.
8. Add 100 µl 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.

VIII. Calculation

Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot.
Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

IX. Related Products:

Salmonella Typhi IgG ELISA Kit (E4678)
Rubella IgM ELISA Kit (E4667)
Toxoplasma IgG ELISA Kit (E4673)
Mumps IgG ELISA Kit (E4669)

FOR RESEARCH USE ONLY! Not to be used on humans.