**Product Information**

*Brucella IgG ELISA*

Catalog Number SE120151  
Storage Temperature 2–8 °C

**TECHNICAL BULLETIN**

**Product Description**

*Brucella* is a Gram-negative coccobacilli capable of infecting humans and a wide range of animals. 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. Worldwide, millions of individual are at risk, especially in developing countries where the infection in animals has not been brought under control, heat treatment procedures of milk (e.g., pasteurization) are not routinely applied, and because of food habits such as consumption of raw milk. The incubation period of brucellosis is usually one to three weeks, but sometimes may be several months. The illness may be mild and self-limiting, or severe. The disease is accompanied by continued, intermittent, or irregular fever, headache, weight loss, and generalized aching and fatigue. Urogenital symptoms may dominate the clinical presentation in some patients.

This method uses *B. abortus* outer membrane, which is shared by the other species. *Brucella* IgG and IgA antibodies persist for many years after infection. A significant increase in *Brucella* IgG level in patients with symptoms of brucellosis is indicative of recent exposure. IgM antibodies are present in acute brucellosis and also found in ~33% of patients with chronic brucellosis.

The *Brucella* IgG ELISA kit is intended for the detection of IgG antibody to *Brucella in human serum or plasma. Diluted serum is added to wells coated with purified antigen. IgG 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 TMB Substrate is added. The plate is incubated to allow the oxidation of the Substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

**Components**

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwell coated with <em>Brucella abortus</em> antigen</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle (ready to use)</td>
<td>22 mL</td>
</tr>
<tr>
<td>Calibrator: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Positive Control: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control: 1 vial (ready to use)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Wash concentrate 20x: 1 bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

**Reagents and Equipment Required but Not Provided.**

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

**Sample Preparation**

1. Collect blood specimens and separate the serum.
2. 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 (18–26 °C).
Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

1. If the O.D. of the Calibrator is >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab index for Positive control should be >1.2.

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.
2. Place the desired number of coated strips into the holder.
3. Negative control, positive control, and calibrator are ready to use. Prepare 21-fold dilution of test samples, by adding 10 µL of the sample to 200 µL of Sample Diluent. Mix well.
4. Dispense 100 µL of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µL of 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 (18–26 °C).
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash Buffer. Blot on absorbent paper or paper towel.
6. Dispense 100 µL of Enzyme Conjugate to each well and incubate for 20 minutes at room temperature.
7. Remove Enzyme Conjugate from all wells. Wash wells three times with 300 µL of 1x Wash Buffer. Blot on absorbent paper or paper towel.
8. Dispense 100 µL of TMB Substrate and incubate for 10 minutes at room temperature (18–26 °C).
9. Add 100 µL of Stop Solution.
10. Read O.D. at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.
Results

Calculations
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. Calculate the cut-off value: Calibrator OD × Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 × 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2/0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6/0.4 = 4.0

Note: Lipemic or hemolyzed samples may cause erroneous results.

Interpretation
The following is intended as a guide to interpretation of Brucella IgG antibody index (Ab Index) test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

<0.9 – No detectable antibody to Brucella IgG by ELISA
0.9–1.1 – Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1 – Detectable antibody to Brucella IgG by ELISA

References

CH,MAM,RGC 10/14-1