# Brucella IgG ELISA Kit

Catalog Number KA0954

96 assays

Version: 02

Intended for research use only

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## Introduction

### Intended Use

The Brucella IgG ELISA Kit is intended for the measurement of IgG antibody to Brucella in human serum or plasma. For research use only.

### Background

Brucella is a 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 patogenic 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 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 is in patients with symptoms of brucellosis is indicative of recent exposure. IgM antibodies are present in acute brucellosis and also found in about 33% of patients with chronic brucellosis.

### Principle of the Assay

Diluted sample 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 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 IgG specific antibody in the sample.

## General Information

### Materials Supplied

List of component

| Component | Amount |
|---|---|
| Microwell coated with Brucella abortus antigen | 12 x 8 x 1 |
| Sample Diluent: ready to use | 22 mL |
| Calibrator: ready to use | 1 mL |
| Positive Control: ready to use | 1 mL |
| Negative Control: ready to use | 1 mL |
| Enzyme conjugate: ready to use | 12 mL |
| TMB Substrate: ready to use | 12 mL |
| Stop Solution: ready to use | 12 mL |
| Wash concentrate 20X | 25 mL |

### Storage Instruction

- ✓ Store the kit at 2-8°C.
- ✓ Keep microwells sealed in a dry bag with desiccants.
- ✓ The reagents are stable until expiration of the kit.
- ✓ Do not expose test reagent to heat, sun, or strong light.

### Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450 nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

## Precautions for Use

✓ Warnings and Precautions

- This kit is designed for research use only.
- Potential biohazardous materials:

  The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- 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.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

✓ Limitation of the test:

Lipemic or hemolyzed samples may cause erroneous results.

## Assay Protocol

### 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).

### Sample Preparation

- Collect blood specimens and separate the serum.
- 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.

### Assay Procedure

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

1. Bring all specimens and kit reagents to room temperature (20-25°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 1:21 dilution of test samples, by adding 10 µL the sample to 200 µL sample diluent. Mix well.
4. Dispense 100 µL diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µL 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.
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance 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 absorbance paper or paper towel.
8. Dispense 100 µL of TMB substrate and incubate for 10 minutes at room temperature.
9. Add 100 µL of stop solution.
10. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

## Data Analysis

**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).
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 x 0.5= 0.400

Positive control O.D. = 1.2

Ab Index = 1.2 / 0.4 = 3

Sample O.D. = 1.6

Ab Index = 1.6 / 0.4 = 4.0

✓ Quality Control

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

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

✓ Interpretation

The following is intended as a guide to interpretation of Brucella IgG ELISA Kit results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

✓ Antibody Index Interpretation

<0.9 No detectable antibody to Brucella IgG by ELISA.

0.9-1.1 Borderline positive.

>1.1 Detectable antibody to Brucella IgG by ELISA.

Performance Characteritics

✓ Sensitivity and Specificity

92 patient sera were tested by this Brucella IgG ELISA Kit and a reference ELISA method. 14 sera were positive and 77 were negative by both methods (99% agreement). The results are summarized below:

| | Brucella IgG ELISA Kit | | |
|---|---|---|---|
| | + | - | Total |
| Reference ELISA Kit + | 14 | 0 | 14 |
| - | 1 | 77 | 78 |
| Total | 15 | 77 | 92 |

✓ Precision

Intra Assay Study

| Serum | No. of Replicates | Mean | Standard Deviation | Coefficient of Variation % |
|---|---|---|---|---|
| 1 | 16 | 1.31 | 0.071 | 5.41 |
| 2 | 16 | 0.86 | 0.052 | 6.04 |
| 3 | 16 | 0.24 | 0.015 | 6.25 |

Inter Assay Study

| Serum | No. of Replicates | Mean | Standard Deviation | Coefficient of Variation % |
|---|---|---|---|---|
| 1 | 10 | 1.92 | 0.21 | 10.93 |
| 2 | 10 | 1.44 | 0.17 | 11.80 |
| 3 | 10 | 0.25 | 0.032 | 12.80 |

## Resource

### Reference


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3. Bowden RA; Cloeckaert A; Zygmunt MS; Bernard S; Dubray G. Surface exposure of outer membrane protein and lipopolysaccharide epitopes in Brucella species studied by enzyme-linked immunosorbent assay and flow cytometry. Infect Immun 1995; 63(10):3945-52.
4. Baldi PC; Miguel SE; Fossati CA; Wallach JC. Serological follow-up of human brucellosis by measuring IgG antibodies to lipopolysaccharide and cytoplasmic proteins of Brucella species. Clin Infect Dis 1996;22(3):446-55
5. Casao MA; Leiva J; Diaz R; Gamazo C. Anti-phosphatidylcholine antibodies in patients with brucellosis. J Med Microbiol 1998; 47(1):49-54.

**Plate Layout**

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