Brucella

IgG – ELISA

Enzyme immunoassay for the qualitative determination of IgG-class
antibodies against Brucella
in human serum
Only for in-vitro diagnostic use

Product Number: BRUG0050 (96 determinations)

CONTENTS

1. INTRODUCTION

Brucella is a small Gram-negative bacterium (0.4-0.8 µm in diameter and 0.4-3.0 µm in length) which is non-flagellated, and non-spore-forming. Since the discovery of Brucella melitensis by Bruce in 1887, an increasingly complex pattern of strains has emerged, and each type has distinctive epidemiological features. Virulent Brucella organisms can infect both nonphagocytic and phagocytic cells; the mechanisms of pathogenesis of Brucellosis in its natural host species and in humans are still not completely understood.
Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. Although reported incidence and prevalence of the disease vary widely from country to country (from <0.01 to >200 per 100,000 population), bovine brucellosis caused mainly by B. abortus is still the most widespread form. Risk groups include abattoir workers, meat inspectors, animal handlers, veterinarians, and laboratorians. Brucellosis is a nationally notifiable disease and reportable to the local health authority.

Species	Disease	Mechanism of Infection
B. abortus (cattle) B. metitensis (sheep,goats) B. suis (pigs) B. canis (dogs) B. maris	Acute form: Nonspecific and "flu-like" undulant form: Undulant fevers, arthritis, and orchiepididymitis in young male Chronic form: Chronic fatique syndrome-like, depressive episodes	Zoonotic. Commonly transmitted through abrasions of the skin from handling infected mammals but also by consumption of contaminated milk and dairy products

The presence of infection may be identified by

- Histology; Enzymology: Brucella species may be speciated by susceptibility to the dyes fuchsin
- and thionin, requirement for CO 2 and H 2 S production.
- Serology: Detection of antibodies by ELISA

2. INTENDED USE

The NovaTec Brucella IgG-ELISA is intended for the qualitative determination of IgG class antibodies against Brucella in human serum.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgG-class antibodies against Brucella is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiter strip wells are precoated with Brucella abortus antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled anti-human IgG conjugate is added. This conjugate binds to the captured Brucella-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Brucella-specific IgG antibodies in the specimen. Sulfuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Brucella Coated Wells (IgG):** 12 breakapart 8-well snap-off strips coated with Brucella abortus antigen; vacuum sealed, in resealable aluminium foil.
- **IgG Sample Diluent** ***: 1 bottle containing 100 ml of buffer for sample dilution; pH 7.2 ± 0.2; colored yellow; ready to use; white cap.
- **Stop Solution**: 1 bottle containing 15 ml sulfuric acid, 0.2 mol/l; ready to use; red cap.
- **Washing Solution (20x conc.)*:** 1 bottle containing 50 ml of a 20-fold concentrated buffer (pH 7.2 ± 0.2) for washing the wells; white cap.
- **Brucella anti-IgG Conjugate**:** 1 bottle containing 20 ml of peroxidase labelled rabbit antibody to human IgG; colored red, ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.
- **Brucella IgG Positive Control***:** 1 bottle containing 2 ml; colored yellow; ready to use; red cap.
- **Brucella IgG Negative Control***:** 1 bottle containing 2 ml; colored yellow; ready to use; blue cap.

* contains 0.01 % Thimerosal after dilution
** contains 0.2 % Bronidox L
*** contains 0.1 % Kathon

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and controls to room temperature (20…25°C) before starting the test run!

6.1. Coated snap-off strips

The ready to use breakapart snap-off strips are coated with Brucella antigen. Store at 2...8°C. The strips are vacuum sealed. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the dessiccant supplied and stored at 2...8 °C; stability until expiry date.*

6.2. Brucella anti-IgG Conjugate

The bottle contains 20ml of a solution with anti-human-IgG horseradish peroxidase, buffer, stabilizers, preservatives and an inert red dye. The solution is ready to use. Store at 2...8°C. *After first opening stability expiry date when stored at 2…8°C.*

6.3. Controls

The bottles labelled with Positve and Negative Control contain a ready to use control solution. It contains 0.1% Kathon and has to be stored at 2...8°C. *After first opening stability expiry date when stored at 2…8°C.*

6.4. IgG Sample Diluent

The bottle contains 100ml phosphate buffer, stabilizers, preservatives and an inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8°C. *After first opening stability expiry date when stored at 2…8°C.*

6.5. Washing Solution (20xconc.)

The bottle contains 50ml of a concentrated buffer, detergents, stabilizers and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for at least four weeks if stored at 2...8°C. *Crystals in the solution disappear by warming up to 37 °C in a water bath.*

6.6. TMB Substrate Solution

The bottle contains 15ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. *The solution should be colourless or have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be discharged. After first opening stability expiry date when stored at 2…8°C.*

6.7. Stop Solution

The bottle contains 15ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.
After first opening stability until expiry date..

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum samples with this assay. If the assay is performed within 24 hours after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex. *Positive and negative controls are ready to use and must not be diluted.*

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
2 wells	(e.g. B1+C1)	for the negative control and
1 well	(e.g. D1)	for the positive control.

It is recommended to determine controls and patient samples in duplicate, if necessary.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each control and sample.

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl Brucella anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 30 min at room temperature.** *Do not expose to direct sunlight.*
7. Repeat step 4.

8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 15 min at room temperature in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
    Any blue color developed during the incubation turns into yellow.

    Note: *Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.*
11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA Microwell Plate 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 patient 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.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance value **lower than 0.100.**
- **Negative control** in B1 and C1: Absorbance value **lower than 0.300.**
- **Positive control** in D1: Absorbance value equal to or greater than the cut-off value.

9.2. Calculation of Results

The cut-off is calculated by addition of 0.25 absorbance units to the measured absorption of the mean value of the two negative control determinations.

Example: 0.12 OD neg. control + 0.14 OD neg. control = 0.26 ÷ 2 = 0.13

Cut-off = absorbance mean value of the negative control + 0.25

Cut-off = 0.13 + 0.25 = 0.38

9.3. Interpretation of Results

Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative
→ **grey zone**
It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

9.3.1. Results in NovaTec Units

$$\frac{\text{Patient (mean) absorbance value x 10}}{\text{Cut-off}} = \text{[NovaTec-Units = NTU]}$$

Example: $$\frac{1.786 \times 10}{0.38} = 47 \text{ NTU (NovaTec Units)}$$

Cut-off :	10	NTU
Grey zone:	9-11	NTU
Negative:	<9	NTU
Positive:	>11	NTU

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

Interassay	n	Mean	Cv (%)
Pos. Serum	19	1.85	7.9

Intraassay	n	Mean	Cv (%)
Pos. Serum	7	1.85	6.8

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.
In immunocompromised patients and newborns serological data only have restricted value.

12. PRECAUTIONS AND WARNINGS

- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING:	Thimerosal is toxic! Do not swallow. Avoid contact with skin and mucous membranes!
WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulfuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13. LITERATURE

Salata RA, Ravdin JI. Brucella species (brucellosis).In: Mandel GL, Douglas RG, Bennet JE, eds. Principles and practice of infectious diseases. New York: John Wiley, 1985: 1283-90

AL-Eissa Y, Al-Zamil F, AL Mugeiren M, et al. Childhood brucellosis: a deceptive infectious disease. Scand J Infect 1991; 23: 129-33

Canova CR, Brunner W, Reinhart WH. Brucellose: Fallbericht und Zusammenstellung von 10 Fällen (1973-1992) am Kantonspital Chur. Schweiz Med Wochenschr 1993; 123: 2370-7

McLean DR, Russel N, Khan MY. Neurobrucellosis: clinical and therapeutic features. Clin Infect Dis 1992; 15: 582-90

Robson JM, Harrison MW, Wood RN, et al. Brucellosis: re-emergence and changing epidemiology in Queensland. Med J Aust 1993; 159: 153-8

Al-Eissa Y, Kambal AM, Abrabeeah AA, et al. Osteoarticular brucellosis in children. Ann Rheum Dis 1990; 49:896-900

14. ORDERING INFORMATION

Prod. No.: BRUG0050 Brucella IgG-ELISA (96 Determinations)

SCHEME OF THE ASSAY

Brucella IgG-ELISA

Test preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Sample (diluted 1+100)
Negative control	-	100µl	-	-
Positive control	-	-	100µl	-
Sample (diluted 1+100)	-	-	-	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well three times with 300µl of washing solution				
Conjugate	-	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for 30 min at room temperature** Wash each well three times with 300µl of washing solution				
TMB Substrate	100µl	100µl	100µl	100µl
Incubate for exactly 15 min at room temperature in the dark				
Stop Solution	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)				

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