MONOSCREEN Ab ELISA

Instruction manual
BIOK140-BRUC_NO_(EN)_VA
10/04/2025

Monoscreen AbELISA Brucella

Reference : BIO K 140

ELISA Test ELISA for serodiagnostic of brucellosis

Monowell, indirect test

For veterinary *in vitro* only

Sample	Species	Individual analysis	Pool analysis *, possible up to
Serum	Bovine	✓	10

* This is done in accordance with the legislation in force in your country, the certifying body or the recommendations made by the NRL when they exist. Mixtures must be made volume to volume, i.e. by taking the same volume of each of the sera making up the mixture.

Presentation

Product reference	BIO K 140/5
Format	5 plates, strips of 8 wells
Reactions	480 tests

Composition of the kit

Provided material		BIO K 140/5
Microplates	Microplaque	5
Washing solution	Washing solution (20X)	1 x 250 mL
Dilution solution	Colored dilution solution (1X)	3 x 250 mL
TMB solution	Single component TMB (1X)	1 x 55 mL
Stop solution	Stopping solution (1X)	1 x 55 mL
Conjugate	Conjugate (50X)	1 x 1,5 mL
CTL POS	Positive Control	1 x 0,5 mL
CTL NEG	Negative Control	1 x 0,5 mL

Revision history

Date	Version	Modifications
10/04/2025	V01	Creation

Note : minor typographical, grammar and formatting changes are not included in the revision history.

A. Introduction

Brucellosis is a disease caused by the pathogen *Brucella sp.* It is a bacterium of the order Rhizobiales. Cases of animal brucellosis due to *Brucella melitensis* infections are observed in the Mediterranean basin and the Middle East. *Brucella abortus* is ubiquitous, while cases of *Brucella suis* are mainly found in America, Asia and Oceania.

Animals are infected through ingestion of contaminated products and aerosols. Vertical transmission is also observed during suckling by the contaminated mother. Unlike many bacteria, such as *Salmonella* or *Listeria, Brucella* infection is often asymptomatic. However, *Brucella* induces chronic inflammation that can lead to hygromas, causing joint pain. In males, the bacteria are excreted in the semen, inducing inflammation of the testis or epididymis, leading to orchitis or epididymitis respectively. Complications of the disease can lead to infertility in infected males. The main consequence of *Brucella* infection in pregnant females is abortion. Numerous lesions with necrotic tissue are observed in the placentas of infected pregnant animals.

In particular, the bacterium localizes in the reproductive system and fetal tissues, leading to in utero infections of the offspring, as well as high bacterial levels in placentas and fetal fluids, leading to new infections during parturition or abortion by consumption and aerosol.

B. Test principle

96-well microplates were sensitized with a synthetic *Brucella* antigen. Sera and controls were diluted in the dilution solution. After 60 minutes of incubation and a washing step, the operator adds the conjugate, peroxidase-coupled protein G. After a second 60-minute incubation and a second wash, the chromogen tetramethylbenzidine (TMB) is added. This chromogen has the dual advantage of being more sensitive than other peroxidase chromogens, and of not being carcinogenic.

If *Brucella*-specific immunoglobulins are present in the serum, the conjugate remains bound to the cup containing the *Brucella* antigen, and the enzyme catalyzes the transformation of the colorless chromogen into a blue product. The intensity of staining is proportional to the level of specific antibodies present in the sample.

C. Material required but not provided

- Distilled/demineralized water.
- Dilution microplates.
- Graduated mono or multichannel pipettes (2-20 µL, 20-200 µL and 10-1000 µL range) and single-use tips.
- Microplate washer (optional).
- Microplate reader (450 nm filter).
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...
- Dilution microplate.

Additional kit

- **Tracer : Reference material for Brucellosis serology by ELISA (Ref. : BDE K 140) available on request.**

D. Warnings and precautions of use

- The reagents must be kept between +2 and +8°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Do not use reagents from other kits.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

E. Preparation of the solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Bring the vial to 21±3°C to make sure that all crystals have disappeared; mix the solution well and withdraw the necessary volume.
- The dilution solution is ready to use. The dilution solution is colored yellow. It is used for the dilution of samples, kit controls, tracer and conjugate.
- The conjugate must be diluted 50-fold in the dilution solution.
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Sample preparation

- Sera samples and kit controls (positive and negative) are to be diluted 1:100 in the dilution solution and homogenized. Avoid using hemolyzed or coagulated samples.

Recommended two-step dilution:

1) *10 µL sample + 90 µL dilution solution in dilution microplate.*
2) *10 µL of first step + 90 µL of dilution solution in test plate*

G. Procedure

- Bring all the reagents to 21±3°C before use.
- Carefully read through the previous points.

1. Distribute the **diluted serum samples** and the **diluted kit controls** at **100 µL per well**. Cover and incubate plate at **21 ± 3°C** for **60 ± 5 min**.
2. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL of washing solution per well**. Avoid the formation of bubbles in the wells and the desiccation of the microplate between each wash.
3. Add **100 µL** of **diluted conjugate** per well. Cover and incubate plate at **21 ± 3°C** for **60 ± 5 min**.
4. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL of washing solution per well**. Avoid the formation of bubbles in the wells and the desiccation of the microplate between each wash.
5. Distribute **100 µL** of **TMB solution** per well. Incubate at **21 ± 3°C** for **10 ± 1 min** away from the light, without covering.
6. Distribute the **stopping solution** at a rate of **100 µL per well**. Color changes from blue to yellow.
7. Record optical densities using a plate spectrophotometer with a **450 nm filter** within **5 minutes** of adding the stopping solution.

H. Validation of results

The test can only be **validated** if:

- The difference between positive and negative control optical density readings is greater than 0,600.

$$OD_{\text{positive control}} - OD_{\text{negative control}} > 0{,}600$$

- The positive control gives an optical density higher than 1.65. The negative control one is less than 0.25

I. Interpretation of results

Calculate for each sample its coefficient (S/P %) using the following formula:

$$S/P\,(\%) = \frac{OD\ sample - OD\ negative\ control}{OD\ positive\ control - OD\ negative\ control} * 100$$

	Results	Status
Individual sample	%S/P < 40%	Negative
	%S/P ≥ 40%	Positive
Pool of 10	%S/P < 15%	Negative
	%S/P ≥ 15%	Positive

Get the interpretation of your results quickly and easily using AnalysiScreen, our free online platform, available on our website : https://www.biox.com

ANALYSISCREEN

AnalysiScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. **AnalysiScreen™** is :

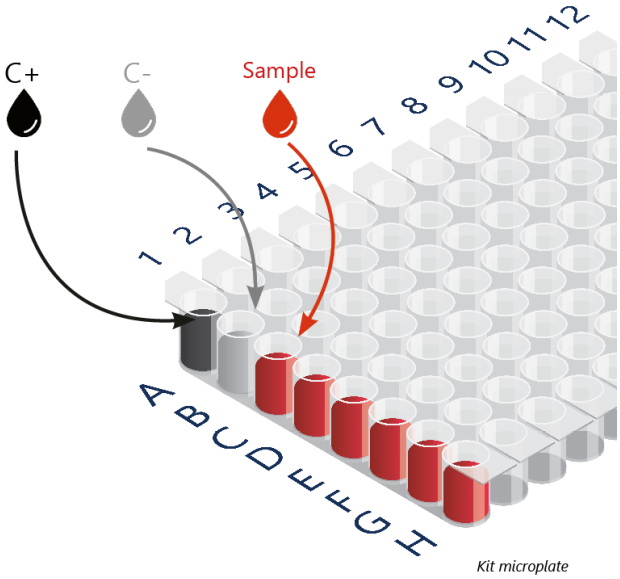
- Free
- Accessible online via our website: https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

SCAN ME

Notes*

1 Distribute 100µL of diluted samples (1/100) and diluted kit controls (positive and negative control) (1/100)

Room Temp. — 60 min → 3X Wash



Kit microplate

2 Add 100 µL of diluted conjugate (1/50)

Room Temp. — 60 min → 3X Wash

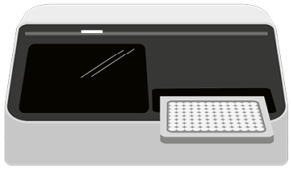
3 Add 100 µL of TMB

Room Temp. — 10 min — Obscurity

4 Add 100 µL of stopping solution

5 Record optical densities

450 nm



* Notes do not replace the instructions of use of which they are a summary.

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