General Information

Indirect ELISA for the detection of antibodies against *Brucella abortus* or *melitensis* in bovine, ovine and caprine milk. The test may be used on individual or bulk milks*.

The assessment of the diagnostic performance of this ELISA kit on field samples has shown that positive bovine milk samples may be detected in pools up to 250.

** Note: The analysis of milk from small ruminants and the analysis of individual milks are not official EU tests.*

Description and Principle

The wells are coated with purified *Brucella abortus* LPS.

Specimens to be tested and the controls are added to the microwells. Anti-*Brucella* antibodies, if present, form an antibody-antigen complex.

An anti-ruminant horseradish peroxidase (HRP) conjugate is added to the microwells. It fixes to the anti-*Brucella* antibodies, forming an antigen-antibody-conjugate-HRP complex.

After washing in order to eliminate the excess conjugate, the Substrate Solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested:

- In the presence of antibodies, a blue solution appears which becomes yellow after addition of the Stop Solution.
- In the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Kit Components

Reagents*
Microplates (strips of 12 x 8 wells) coated with purified *Brucella* LPS
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 3
Wash Concentrate (20X)
Substrate Solution (TMB)
Stop Solution (0.5 M)

**Quantities supplied are indicated on the kit label*

1. The conjugate, the controls and the substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C et +26°C.
3. Components bearing the same name (*wash solution, dilution buffers*) can be used for the entire IDvet product range.

Note: If needed, IDvet can supply you with additional volumes of the above components.

Materials required but not provided

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µl, 100 µl, and 200 µl.
2. Disposable tips.
3. 96-well microplate reader.
4. Distilled or deionized water.
5. Manual or automatic wash system.
6. 96-well pre-dilution microplate.

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Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0.5 M) may be harmful if swallowed. It may cause sensitisation by skin contact (**R22-43**). Avoid contact with skin (**S24-37**).
4. Do not expose the substrate solution to bright light nor to oxidizing agents.
5. All single-use material used for the assays should be decontaminated by immersion in freshly prepared 5% sodium hypochlorite for minimum 1 hour before elimination, or by autoclaving at 120°C.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate is completely solubilised. Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20X)** in distilled/deionised water.

Testing Procedure

Allow all reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or Vortex.

Centrifuge each whole milk sample, or just let the samples sit, so that the cream separates from the lactoserum (cream on the top, lactoserum on the bottom):

Pipette under the cream so that only the lactoserum enters the cone (antibodies are found in the lactoserum).

1. Add:
   - **100 µl** of the Negative Control to wells A1 and B1.
   - **100 µl** of the Positive Control to wells C1 and D1.
   - **100 µl** of each milk or pooled samples to be tested to the remaining wells.
2. Incubate **45 min** ± 4 min at 21°C (± 5°C) (short incubation) or **overnight** (for 16 to 20 hours) at 4°C (± 2°C).
3. Empty the wells. Wash each well 3 times with approximately 300 µl of the **Wash Solution**. Avoid drying of the wells between washings. *Be careful that there is no fatty ring left in the well after washing. To avoid fat residues, it is possible to include a soaking time of 2 – 5 minutes between washes.*
4. Prepare the **Conjugate** by diluting the **Concentrated Conjugate 10X** to 1/10 in **Dilution Buffer 3** (short incubation) or to 1/20 (overnight incubation) in **Dilution Buffer 3.**
5. Add 100 µl of the **Conjugate 1X** to each well.
6. Incubate **30 min** ± **3 min at 21°C (± 5°C).**
7. Empty the wells. Wash each well 3 times with approximately 300 µl of the **Wash Solution**.
8. Add 100 µl of the Substrate Solution to each well.
9. Incubate 15 min ± 2 min at 21°C (±5°C) in the dark.
10. Add 100 µl of the Stop Solution to each well in order to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean corrected value of the Positive Control OD (OD_{PC}) is greater than 0.350.

$$OD_{PC} > 0.350$$

✓ the ratio of the mean corrected values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is greater than 3.

$$OD_{PC} / OD_{NC} > 3$$

Interpretation

For each sample, calculate the S/P percentage as follows using the corrected sample and control values:

$$S/P = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

For short and overnight incubation

Samples with a S/P:

- less than or equal to 45% are considered negative.
- greater than 45% and less than or equal to 50% are considered doubtful.
- greater than 50% are considered positive

Result	Status
S/P % ≤ 45%	NEGATIVE
45% < S/P % ≤ 50%	DOUBTFUL
S/P % > 50%	POSITIVE

Certified management system

ID Screen®
Brucellosis Milk Indirect

Indirect ELISA for the detection of antibodies against *Brucella abortus or melitensis* in bovine, ovine and caprine milk.

Individual or bulk milks.

Short and overnight incubation

For *in vitro* use

April 2015 :

Controls and samples are added undiluted to the wells

November 2012 :

Overnight incubation was added

BRUMILK ver 0415 EN

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