

SERELISA[®] Brucella OCB Ab Mono Indirect

KIT FOR THE DETECTION OF ANTI *Brucella*
LIPOPOLYSACCHARIDE ANTIBODIES
IN BOVINE SERA (INDIVIDUAL AND POOLS),
OVINE AND CAPRINE SERA (INDIVIDUAL)

INDIRECT IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The "SERELISA[®] Brucella OCB Ab Mono Indirect" kit uses an indirect immunoenzymatic technique allowing the detection of *Brucella* lipopolysaccharide (LPS) antibodies in individual bovine, ovine and caprine serum samples or pools of 10 bovine sera (in accordance with applied regulations). The reaction is composed of three steps:

- Each individual or pooled serum sample is placed in a well sensitised with the Brucella LPS. Antibodies present in the sample bind to the bacterial antigen coated to the wells.
- After a wash step, a peroxidase conjugate is added. It fixes to the immunoglobulins (antibodies) previously captured, forming a complex:
(Ag LPS) - (Ab anti-LPS) - (peroxidase conjugate)
- Excess conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by addition of a substrate, which is transformed into a coloured product. After stopping the reaction, the optical densities are measured. The presence or absence of antibodies is determined using threshold values obtained from the positive control.

II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
4 microplates containing 6 strips of 2 x 8 wells sensitised with Brucella LPS	Use within 4 weeks after opening of the sachet, which must be closed after use.
Conjugate: peroxidase conjugate (CJ)	<i>Individual sera protocol:</i> Dilute 200 times in the CD. <i>Pool sera protocol:</i> Dilute 100 times in the CD. Use within 2 h after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Negative control (N)	<i>Individual sera protocol:</i> Dilute 100 times in the SD. <i>Pool sera protocol:</i> Dilute 200 times in the SD.
Positive control (P)	<i>Individual sera protocol:</i> Dilute 100 times in the SD. <i>Pool sera protocol:</i> Dilute 200 times in the SD.
Sample diluent (SD)	Ready-to-use.
Wash solution (W) (10X concentrated)	Dilute 10 times in distilled or demineralised water. Use within 48 hrs after dilution.
Conjugate diluent (CD)	Ready-to-use.
Stop solution (S)	Ready-to-use.
Adhesive films	12 films

Note: Store diluted reagents at +5°C ± 3°C and use as mentioned above.

III. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- Distilled or demineralised water.
- Adjustable or set pipettes to measure and deliver between 0 to 1000 µl. Measurement deviation must be ≤ 10% for volumes ≤10 µl and ≤ 5% for all other volumes.
- Graduated cylinders (100 ml and 1000 ml).
- Manual, automatic or semi-automatic washing device for microtitration plates.
- Microplate reader, fitted with filters for bichromatic reading at 450 and 630 nm. It is also possible to use a monochromatic reader fitted with a 450 nm filter.

IV. PRECAUTIONS FOR USE

The quality of the results depends on the respect of good laboratory practices and the procedure (see paragraph VI).

- Do not mix or associate reagents from kits with different batch numbers
- Do not use reagents after the expiry date.
- Place all reagents at laboratory temperature for at least 1 hour prior to use. **Caution:** only the reagents to be used in the following step are concerned.
- Handle all reagents and samples as biohazardous material.
- Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
- Never pipette by mouth.
- Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
- Avoid contamination of the substrate solution with metallic ions, oxidizing agents or detergents. Make sure that all containers are clean. Do not use the same container or the same pipette tip for the conjugate and the substrate.
- It is recommended to dispose reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

Risk phrases:

- R 23/25: Toxic by inhalation and if swallowed.
R35: Causes severe burns.
R 36/37/38: Irritating to eyes, respiratory system and skin.
R 41: Risk of serious damage to eyes.
R 42/43: May cause sensitisation by inhalation and skin contact.
S 7: Keep container tightly closed.
S 24: Avoid contact with skin.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S30: Never add water to the product.
S45: In case of accident or if you feel unwell, seek medical advice immediately.

V. SAMPLES

The reaction is performed on individual sera diluted at 1:100 or 10 pooled samples diluted at 1:20. Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Individual or pooled serum (undiluted)	max. 7 days	Yes	No

VI. PROCEDURE

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each test run, for each plate.

A. PRELIMINARY STEPS

- Carefully set up the distribution and identification of controls and samples.
- Prepare the samples to be tested:

Individual samples protocol

dilution 1:100:

For example, dilute the samples at 1:10 in sample diluent (SD) in a test tube or into a dilution plate. Dilute again at 1:10 in a test tube or directly

into the wells: dispense 10µl of already diluted sample in 90µl of sample diluent (SD).

Pooled bovine samples protocol **dilution 1:20:**

Add for example 10 µl of each individual serum to obtain 100 µl of 10-pooled sample.

Dilute pooled samples at 1:20 in a test tube or directly into the wells: dispense 5µl of pooled samples in 95µl of sample diluent (SD).

B. TEST PROCEDURE

I - CONTROL AND SAMPLE DISTRIBUTION

1. Control distribution:

For the two following protocols, 100 µl of the diluted negative control (N) is distributed in wells A1 and A2, 100 µl of the diluted positive control (P) in wells B1 and B2.

Individual samples protocol **dilution 1:100:**

After shaking the vials, dilute for example the controls at 1:10 in sample diluent (SD) in a test tube or into a dilution plate then dilute again at 1:10 in a test tube: dispense 10µl of already diluted control in 90µl of sample diluent (SD).

Pooled bovine samples protocol **dilution 1:200:**

After shaking the vials, dilute the controls at 1:10 in sample diluent (SD) in a test tube or into a dilution plate then dilute again at 1:20 in a test tube or directly into the wells: dispense 5µl of already diluted control in 95µl of sample diluent (SD).

2. Sample distribution:

Strictly comply with the procedure indicated in VI.A.2. for the preparation of samples and the distribution directly in the plate.

- The samples can be tested individually or in duplicate. Distribute 100 µl in each well.
- Strips should always be placed on the frame so that both washer and reader can be used.
- Cover the wells with adhesive film, cut to the necessary length by the number of strips used.
- Mix by gentle shaking the plate manually or by using a plate agitator.

3. Incubation of the plate

1 hour (± 5 min) at + 37°C (± 3°C)

WASHING:

Wash buffer: dilute the concentrated washing solution (W) 1:10 in distilled or demineralised water.
Carefully remove the adhesive film and wash 4 times:

II – ADDITION OF CONJUGATE

1. Preparation of conjugate:

Individual samples protocol **dilution 1:200:**

Dilute the concentrate (CJ) 1:200 in the conjugate diluent (CD). 2 ml are needed for one strip, meaning 10 µl of CJ in 1.99 ml of CD.

Pooled samples protocol **dilution 1:100:**

Dilute the concentrate (CJ) 1:100 in the conjugate diluent (CD). 2 ml are needed for one strip, meaning 20 µl of CJ in 1.98 ml of CD.

2. Distribution of conjugate:

Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

3. Incubation of conjugate:

Incubate 30 minutes (± 5 min) at +37°C (± 3°C).

WASHING:

Carefully remove the adhesive film and wash 4 times.

III – REVELATION

1. Addition of the substrate:

Add 100 µl of buffered peroxidase substrate (PS) per well. Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually or use a plate agitator to ensure correct homogenisation.

2. Incubation of substrate:

30 min. ± 5 min. at laboratory temperature (+20°C ± 5°C), shielded from light.

3. Addition of Stop Solution:

Add 50 µl of stop solution (S) per well.

Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells.

4. Measure of the optical density:

Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).

Reading bichromatically is strongly recommended. Should a monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading.

VII. TEST VALIDATION

The results of each test run are valid if:

$$\overline{OD} P \geq 0.5 \text{ and } \overline{OD} N < 0.3 * \overline{OD} P$$

\overline{OD} : average of the ODs for the samples tested in duplicate.

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

The presence or the absence of antibodies against LPS of *Brucella* is determined by comparing the Optical Densities (OD) to the threshold values obtained from the positive control.

Two methods for calculating and interpreting the results are possible:

Method 1: INDEX CALCULATION:

Positive threshold value in index = 0

For individual and pooled samples:

$$\text{Sample index} = 0.50 \times (\text{sample OD} - 0.6 \times \overline{OD} P)$$

Any sample or pool of samples presenting an index ≥ 0 is considered as **positive**.
Any sample or pool of samples presenting an index < 0 is considered as **negative**.

Method 2: ANALYSIS OF THE OPTICAL DENSITIES

For individual and pooled samples:

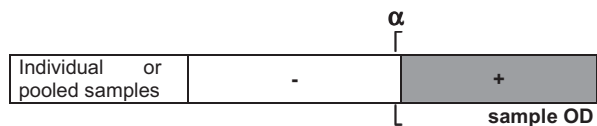
$$\text{Positive threshold value} \Rightarrow \alpha = 0.6 \times \overline{OD} P$$

Compare each sample OD to this threshold value.

Any individual or pooled sample presenting an $OD \geq \alpha$ is considered as **positive**.

Any individual or pooled sample presenting an $OD < \alpha$ is considered as **negative**.

Result interpretation



Should you have any question, please contact us:
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