

LACTELISA[®] Brucella Ab Mono Indirect

KIT FOR THE DETECTION OF ANTI BRUCELLA ANTIBODIES IN BOVINE MILK SAMPLES (INDIVIDUAL AND POOLS)

INDIRECT IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The LACTELISA[®] Brucella Ab Mono Indirect kit uses a single well indirect immunoenzymatic technique for the detection of anti-Brucella specific antibodies in individual or pooled milk samples, according to the applicable country legislation.

The reaction is composed of 3 steps:

- Each milk sample is placed in a well coated with lipopolysaccharides (LPS) of *Brucella*. Antibodies present in the sample will bind to the LPS of Brucella coated to the wells.
- After a wash step, an anti-bovine IgG monoclonal antibody (Mab) / peroxidase conjugate is added. It fixes onto present bovine immunoglobulins (antibodies), forming a complex:
(Ag)-(Ab)-(anti bovine IgG Mab / peroxidase).
- Excess of free conjugate is eliminated by a second wash step. The enzyme linked to the conjugate is revealed by addition of a substrate which transforms it into a coloured product. After stopping the enzymatic reaction, the optical densities are read. The presence or absence of antibodies is determined by using threshold values obtained through the use of 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 LPS of Brucella	Use within 4 weeks after opening of the sachet which must be closed after use.
Conjugate : anti-bovine IgG Mab / peroxidase (CJ) (20 X concentrated)	Dilute 20 times in the conjugate diluent and use within 24 hrs after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Negative control (N)	Ready-to-use.
Positive control (P)	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: Kit and diluted reagents should be stored at + 5°C ± 3°C and used 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.
- Incubator at +37°C ± 3°C.

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.
- 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:

- R35: Causes severe burns.
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 or pooled (up to 50 milks) non-diluted, skimmed milks.

Technique for total skimming: centrifuge milk samples at a velocity that enables the separation between the lipidic layer and lactoserm.

Technique for partial skimming: for milk decantation, wait sufficiently to permit the separation of lipidic layer and lactoserm.

Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Individual or pooled milk	max. 5 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. Milk samples should not be diluted. Skimming is advised but not necessary. It is recommended to homogenise the sample before pipetting.

B. TEST PROCEDURE

I - CONTROL AND SAMPLE DISTRIBUTION

1. Control distribution:

Controls are ready-to-use.

After shaking the vials, add 200 µl of negative control (N) to wells A1 and A2, and 200 µl of positive control (P) to wells B1 and B2.

2. Sample distribution:

Dispense 200 µl of the non-diluted milk samples in the test wells. The samples can be tested individually or in duplicate

- 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

Incubate the plate either for 1 hour ± 5 min. at +37°C ± 3°C or overnight (14-18 hours) at +5°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:

Dilute the concentrate (CJ) 1:20 in the conjugate diluent (CD). 4 ml are needed for one strip, meaning 200 µl of CJ in 3.8 ml of CD.

2. Distribution of conjugate:

Add 200 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

3. Incubation of conjugate:

Incubate either for 1 hour ± 5 min. at +37°C ± 3°C or for 90 min. ± 5 min. at laboratory temperature (+20°C ± 5°C).

WASHING :

Carefully remove the adhesive film and wash 4 times.

III – REVELATION

1. Addition of the substrate:

Add 200 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:

- the optical density obtained with the positive control ($\overline{OD} P$) is > 0.300, and

- the optical density obtained with the negative control ($\overline{OD} N$) is < 0.25 x OD P.

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:

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

OD: average of the sample optical densities if the test is performed in duplicate.

Calculation of the threshold index values:

For pools: threshold value: $-0.20 \times (\overline{OD} P)$

For individual milks: positive threshold index value : $-0.05 \times (\overline{OD} P)$

negative threshold index value : $-0.20 \times (\overline{OD} P)$.

Any individual milk sample presenting an index $\geq -0.05 \times \overline{OD} P$ is considered as **positive**.

Any individual milk sample presenting an index $< -0.2 \times \overline{OD} P$ is considered as **negative**.

Any individual milk sample presenting an index between $-0.2 \times \overline{OD} P$ and $-0.05 \times \overline{OD} P$ is considered as **doubtful**.

Any pool of milks sample presenting an index $\geq -0.2 \times \overline{OD} P$ is considered as **positive**.

Any pool of milks sample presenting an index $< -0.2 \times \overline{OD} P$ is considered as **negative**.

Method 2 : ANALYSIS OF THE OPTICAL DENSITIES

Calculate the OD threshold values corresponding to $[0.9 \times (\overline{OD} P)]$ (positive value for individual) and $[0.6 \times (\overline{OD} P)]$ (negative value for individuals and positive value for pools).

Compare each of the ODs obtained for the milk samples to these threshold values.

Any individual milk sample showing an OD value $\geq 0.9 \times \overline{OD} P$ is considered as **positive**.

Any individual milk sample showing an OD value $< 0.6 \times \overline{OD} P$ is considered as **negative**.

Any individual milk sample showing an OD value between $0.9 \times \overline{OD} P$ and $0.6 \times \overline{OD} P$ is considered as **doubtful**.

Any pool of milks sample showing an OD value $\geq 0.6 \times \overline{OD} P$ is considered as **positive**.

Any pool of milks sample showing an OD value $< 0.6 \times \overline{OD} P$ is considered as **negative**.

Result interpretation :

	0	$0.60 \times (\overline{OD} P)$	$0.90 \times (\overline{OD} P)$	
Individual milk	-	+/-	+	sample OD
Pooled milk	-	$0.60 \times (\overline{OD} P)$	+	sample OD

Should you have any question, please contact us :
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