

SERELISA[®] M. ParaTB Ab Mono Indirect

**KIT FOR THE DETECTION OF ANTI - MYCOBACTERIUM
AVIUM SUB. PARATUBERCULOSIS ANTIBODIES
IN SAMPLES OF SERUM AND PLASMA FOR CATTLE,
SHEEP, GOATS AND CATTLE MILK
(INDIVIDUAL)**

INDIRECT IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] M. ParaTB Ab Mono Indirect kit uses an indirect immunoenzymatic technique allowing the diagnosis of Johne's disease through the detection of *Mycobacterium avium* sub. *paratuberculosis* (M. ParaTB) antibodies in samples of serum and plasma for cattle, sheep, goat and cattle milk. In order to avoid cross reactions, test samples, and controls are pre-diluted in the sample diluent containing *Mycobacterium phlei*.

The technique used in this kit is described in the recommendations of the OIE (Manual of Recommended Diagnostic Techniques and Requirements volume III - Paratuberculosis 5 B/009).

The reaction is composed of three steps:

1. Each pre-diluted sample is placed in a well sensitised with a purified *Mycobacterium sp.* antigen. Antibodies present in the sample bind to the bacterial antigen coated on the well.

2. After a wash step, a conjugate anti-species / peroxidase conjugate is added. It fixes to the bovine immunoglobulins (antibodies) previously captured, forming a complex:

(Ag) - (Ab anti- M. ParaTB) - (conj anti-species / peroxidase)

3. Excess of conjugate is eliminated by a wash step. The enzyme linked to the complex is revealed by the 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 12 strips of 8 wells sensitised with M. ParaTB. antigen | Use within 2 months after opening of the sachet which must be closed after use. |
| Conjugate : anti-species / peroxidase (CJ) (concentrated) | Dilute in the conjugate diluent to 1/100 for short protocol and 1/200 for long protocol. Use within 2 hrs after dilution. |
| Buffered peroxidase substrate (ABTS) | Ready-to-use. |
| Negative control (N) | Dilute 40 times as sera. |
| Positive control (P) | Dilute 40 times as sera. |
| Sample diluent pink color (SD) | Ready-to-use. |
| Wash solution (W) (20X concentrated) | Dilute 20 times in distilled or demineralised water. Use within 5 days after dilution at room temperature. |
| Conjugate diluent blue color (CD) | Ready-to-use. |
| Stop solution (S) | Ready-to-use. |
| Adhesive films | 12 films |

Note: Kit and diluted reagents should be stored at +5 ± 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 with filters for reading at 405 or 410nm.

IV. PRECAUTIONS FOR USE

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

1. Do not mix or associate reagents from kits with different batch numbers.
2. Do not use reagents after the expiry date.
3. 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
4. Handle all reagents and samples as biohazardous material.
5. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
6. Never pipette by mouth.
7. Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
8. 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.
9. 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 and safety phrases:

R22: Harmful if swallowed.

R36/38: Irritating to eyes and 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.

S36: Wear suitable protective clothing.

V. TREATMENT AND STORAGE OF SAMPLES

The reaction is performed on serum and plasma of cattle, sheep, goat and cattle milk. Milk samples should be skimmed before testing (either overnight decantation or low-speed centrifugation).

The undiluted samples can be stored as follows:

| Samples | Cold (+5°C) | Freeze (-20°C) | Lab Temperature (+23°C) |
|----------------------------|--------------|----------------|-------------------------|
| Serum or plasma individual | max. 7 days | Yes | No |
| Milk individual | 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.

Position negative control (N) in wells A1 and A2 and positive control (P) in wells B1 and B2.

B. TEST PROCEDURE

I - CONTROL AND SAMPLE DILUTION AND DISTRIBUTION

Controls are diluted as serum and plasma samples.

- **SERUMS, PLASMA, CONTROLS (dilution to 1:40)**

The samples, positive (P) and negative (N) controls are pre-diluted in a pre-dilution plate (pre adsorption) to 1:10 then diluted to 1:4:

1. Dilution 1:10, dispense in a pre-dilution plate 10 µl per well of samples and controls then add 90 µl of sample diluent (SD). Mix by gentle shaking the plate manually or by using a plate agitator (pre-dilution plate can be stored 5 days at -20°C).

Incubate in pre-dilution plate between 5 and 20 minutes at laboratory temperature (+23°C ± 5°C).

2. Dilution 1:4, dispense in sensitized wells 75 µl of sample diluent (SD) and transfer 25 µl of the pre-diluted sample and controls. Mix by gentle shaking the plate manually or by using a plate agitator.

- **MILKS (dilution to 1:2)**

Milk samples have to be diluted to 1:2 in sample diluent (SD). Dilutions should be performed in hemolysis tubes, in a pre-dilution plate.

Incubate in pre-dilution plate between 5 and 20 minutes at laboratory temperature (+23°C ± 5°C).

- Place 100 µl of the two-fold diluted milks per well.
- Samples may either 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. It is not necessary for short protocol.

Incubation of the plate

- Short protocol: incubate 30 ± 5 min at room temperature (+23 ± 5°C) or
- Long protocol: overnight (14-18h) at +5 ± 3°C.

WASHING:

Wash buffer: dilute the concentrated wash solution (W) 1:20 in distilled or demineralised water.
Carefully remove the adhesive film and wash 6 times.

II - ADDITION OF CONJUGATE

1. Preparation of conjugate:

a. For short sample incubation: Prepare the conjugate solution by diluting the concentrate **CJ** in the conjugate diluent (**CD**) to dilution **1:100** (2 ml are needed per 2 strips, meaning **20 µl** of **CJ** in **1.980 ml** of **CD**).

b. For overnight incubation: Prepare the conjugate solution by diluting the concentrate **CJ** in the conjugate diluent (**CD**) to dilution **1:200** (2 ml are needed per 2 strips, meaning **10 µl** of **CJ** in **1.990 ml** of **CD**).

2. Distribution of conjugate:

Add 100 µl of diluted conjugate per well.

Cover with a new piece of adhesive film (using an adhesive film is not compulsory).

3. Incubation of conjugate:

Incubate for 30 ± 5 min at laboratory temperature (+23 ± 5°C).

WASHING:

Carefully remove the adhesive film and wash 6 times.

III - REVELATION

1. Addition of the substrate:

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

2. Incubation of substrate:

Incubate 15 ± 3 min at laboratory temperature (+23 ± 5°C), shielded from light.

3. Addition of Stop Solution:

Add 100 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) at **405 or 410 nm**.

Ensure the cleanliness of the bottom of the wells prior to reading.

Plate can be read up to 24h after reaction is stopped.

VII. TEST VALIDATION

The results of each test run are valid:

- The average optical density (OD) value of the positive control (P) is ≥ 0.5 and
- if the average optical density (OD) obtained with the negative control (N) is < 0.300

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

- Calculate for each sample the S/P ratio
- Calculate the average of the OD if samples are tested several times

This S/P is calculated as follows:

$$S/P = \frac{OD(S) - \overline{OD(N)}}{OD(P) - \overline{OD(N)}}$$

\overline{OD} : average of the optical densities

RESULTS

Any **individual** serum/plasma sample presenting an S/P ratio ≥ 0.3 is considered **positive**.

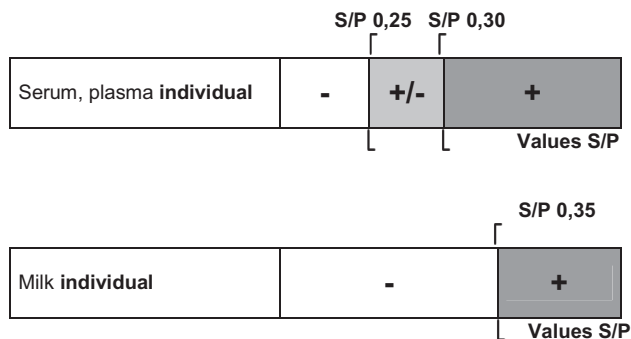
Any **individual** serum/plasma sample presenting an S/P ratio < 0.25 is considered **negative**.

Doubtful Zone:

Any individual serum, plasma which presents a S/P ratio situated in a zone from 0.25 to 0.30 is considered as **doubtful** and should be re-tested. If the doubtful result is confirmed, a second test on a different sample from the same animal is recommended.

Any **milk** samples presenting an S/P ratio ≥ 0.35 is considered **positive**.

Any **milk** samples presenting an S/P ratio < 0.35 is considered **negative**.



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