General Information

This diagnostic kit is designed to detect antibodies against *Mycoplasma bovis*.

It can be used with bovine serum, plasma or milk (individual, pooled, or bulk milk samples).

Description and Principle

Wells are coated with *M. bovis* recombinant antigen.

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

An anti-ruminant horseradish peroxidase (HRP) conjugate is added to the microwells. It fixes to the anti-*M. bovis* 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 coated with *M. bovis* recombinant antigen
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 13
Dilution Buffer 3
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

** Quantities supplied are indicated on the kit label.*

1. The conjugate, controls and substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. Wash, substrate and stop solutions can be used for the entire IDvet product range. Dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 5 µl, 50µl, 100 µl, 200µl and 500 µl.
2. Disposable tips.
3. Distilled or deionized water.
4. Manual or automatic wash system.
5. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitisation by skin contact. Avoid contact with skin and eyes. Use protective lab coat, one-way gloves and safety glasses. The stop solution (0.5 M acid) may be harmful if swallowed.
3. Do not expose the substrate solution to bright light nor to oxidizing agents.
4. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.
5. Please refer to the Material Safety Data Sheet, available upon request at info@id-vet.com , for more detailed information.

Sample Preparation

All samples types

In order to avoid differences in incubation times between specimens, it is possible to prepare a 96-well plate containing the test and control specimens, before transferring them into an ELISA microplate using a multichannel pipette.

Milk samples

This test can be performed on skimmed or whole milk samples, with or without preservatives.
When analysing whole milk samples, special washing precautions should be taken (please refer to "Recommendations for milk testing" available upon request).
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).

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20X)** in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the "IDvet Washing Guide", available upon request.

Testing Procedure

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

It is possible to test in the same run individual serum/plasma and milk samples using the **short incubation protocol.**

However, to increase sensitivity, IDvet recommends using the **overnight incubation protocol** dedicated to milk samples described hereafter. The milk protocol allows for an excellent correlation between paired serum/milk samples.

■ Short incubation protocol:

- **For serum or plasma testing:**

1. Add:
   - 195 µl of **Dilution Buffer 13** to each microwell (for controls, plasma and serum samples)
   - 5 µl of the **Negative Control** to wells A1 and B1.
   - 5 µl of the **Positive Control** to wells C1 and D1.
   - 5 µl of each plasma/serum sample to be tested to the remaining wells.

- **For individual, pooled, or bulk milk samples testing:**

Refer to section "Milk sample preparation".

1. Add:
   - 50 µL of **Dilution buffer 13** to each microwell (for milk testing)
   - 100 µL of milk samples to be tested

- **For all sample types:**

2. Cover the plate and incubate **45 min ± 4 min** at 21°C (± 5°C).

■ Overnight incubation protocol for milk only (individual, pooled or bulk milk samples)

1. Samples are added to the ELISA microplate undiluted and controls at a final dilution of 1:101 as follows:

a) Pre-dilute the **Negative Control** and the **Positive Control** to 1:101 in **Dilution Buffer 13** to generate the **NC milk** and **PC milk** controls using for each control one microtube with 1000µl of buffer and 10µl of control.

b) In the ELISA microplate, add:
   - 50 µl of **Dilution Buffer 13** to each microwell (for controls and samples)
   - 100 µl of the **NC milk** prepared as described hereabove to wells A1 and B1.
   - 100 µl of the **PC milk** prepared as described here above to wells C1 and D1.
   - 100 µl of each milk sample to be tested to the remaining wells.

2. Cover the plate and incubate **overnight (16-20 hours)** at 21°C (± 5°C).

■ For all protocols

3. Empty the wells. Wash each well at least* 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.

   Note: If testing milk samples, 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. Additional washing steps (up to 6) can also be added. These additional washes can also be done when testing serum/plasma samples together with milk samples without affecting test performance on these sample types.

4. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 3.**
5. Add 100 µl of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 min ± 3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
8. Add 100 µl of the **Substrate Solution** to each well.
9. Cover the plate and 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 the same order as in step No. 8, to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

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

$$\mathbf{OD_{PC} > 0.350}$$

✓ the ratio of the mean values of the Positive Control O.D. (OD_{PC}) to the Negative Control O.D. (OD_{NC}) is greater than 3.

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

Interpretation

■ Short incubation protocol

For each sample, calculate the S/P percentage (S/P %):

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

Result	Status
SERUM or PLASMA	
S/P % < 60%	NEGATIVE
S/P % ≥ 60%	POSITIVE
INDIVIDUAL, POOLED or BULK MILK	
S/P % < 20%	NEGATIVE
S/P % ≥ 20%	POSITIVE

If requested, positive results can be classified as follows:

Result		*Status*
SERUM or PLASMA	***MILK***	
60%≤S/P %<80%	*20%≤S/P %<40%*	+
80%≤S/P %<110%	*40%≤S/P %<60%*	++
110%≤S/P %<140%	*60%≤S/P %<80%*	+++
S/P % ≥ 140 %	*S/P % ≥ 80 %*	++++

■ Overnight incubation protocol (milk only)

For each milk sample, calculate the S/P percentage (S/P_{milk} %)

$$\boldsymbol{S/P\,milk\,\% = \frac{OD_{sample} - OD_{NCmilk}}{OD_{PC\,milk} - OD_{NCmilk}} \times 100}$$

INDIVIDUAL, POOLED or BULK MILK	
Result	Status
S/P_{milk} % < 30%	NEGATIVE
S/P_{milk} % ≥ 30%	POSITIVE

If requested, positive results can be classified as follows:

Result	*Status*
30%≤S/P %<50%	+
50%≤S/P %<100%	++
100%≤S/P %<150%	+++
S/P % ≥ 150 %	++++

Note: The IDSoft™ data analysis program is available free-of-charge. Please contact support.software@id-vet.com for more information. This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested).

IDvet
Innovative Diagnostics

Certified management system

ID Screen®
Mycoplasma bovis Indirect

Indirect ELISA for the detection of antibodies against *Mycoplasma bovis* in bovine serum, plasma or milk

For *in vitro* use

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