General Information

This diagnostic kit is designed to detect antibodies against *Mycoplasma agalactiae*, responsible for contagious agalactia in small ruminants.

It can be used with individual ovine or caprine serum and plasma.

Description and Principle

Wells are coated with purified *M. agalactiae* P48 recombinant antigen.

Samples to be tested and controls are added to the microwells. Anti-P48 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-P48 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 coloration 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. agalactiae* P48 recombinant antigen
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 2
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 10 µl, 100 µl, 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.

Please refer to the Material Safety Data Sheet, available upon request at info@id-vet.com, for more detailed information.

Sample Preparation

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.

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)** to 1/20 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.

1. Add:
   - 190 µl of **Dilution Buffer 2** to each microwell.
   - 10 µl of the **Negative Control** to wells A1 and B1.
   - 10 µl of the **Positive Control** to wells C1 and D1.
   - 10 µl of each sample to be tested to the remaining wells.
2. Cover the plate, **shake on an orbital plate shaker (i.e : 1 min at 500 rpm) or shake manually**, and incubate **45 min ± 4 min** at 21°C (± 5°C).
3. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
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 and Negative Controls O.D. (OD_{PC} and OD_{NC}) is greater than 3.

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

Interpretation

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

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

Samples presenting a S/P%:

- less than or equal to 50% are considered negative.
- greater than 50% and less than 60% are considered doubtful.
- greater than or equal to 60 % are considered positive.

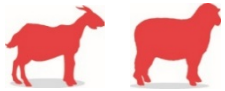
Result	Status
S/P % ≤ 50%	NEGATIVE
50% < S/P % < 60%	DOUBTFUL
S/P % ≥ 60%	POSITIVE

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).

Certified management system

ID Screen®
Mycoplasma agalactiae Indirect



Indirect ELISA for the detection of antibodies against *Mycoplasma agalactiae* in individual ovine or caprine serum and plasma.

For *in vitro* use

MAGALS ver 0219 EN

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IDvet, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com