General Information

This indirect ELISA diagnostic kit is designed to detect antibodies directed against *P. multocida* in duck serum

Description and Principle

Microwells are coated with *P. multocida* antigen extract.

Samples to be tested and controls are added to the wells. Anti-*Pasteurella multocida* antibodies, if present, form an antigen-antibody complex.

After washing, an anti-duck horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the sample antibodies, forming an antigen-antibody-conjugate-HRP complex.

After elimination of the excess conjugate by washing, 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 *P. multocida* antigen
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 14
Dilution Buffer 3
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0,5 M)

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

1. The conjugate, the controls and the substrate solution must be stored at 5°C (± 3°C).
2. Other reagents can be stored between +2°C and +26°C.
3. Components bearing the same name (*wash solution, dilution buffers*) can be used for the entire IDvet product range.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 5 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, 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 **(20X)** 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 at info@id-vet.com.

Testing Procedure

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

The negative and positive controls are supplied ready-to-use. DO NOT add dilution buffer to the control wells A1, B1, C1 and D1 – the controls are to be tested un-diluted.

Samples, however, are tested at a **final dilution of 1:50 in Dilution Buffer 14.**

1. In a pre-dilution plate, set aside wells A1, B1, C1 and D1 for the controls, and add:
   - 5 µl of each sample to be tested,
   - 245 µl of **Dilution Buffer 14** to all wells EXCEPT control wells A1, B1, C1 and D1.

Note: It is recommended to respect the indicated order of deposit to be able to visually control sample presence in each well.

2. In the ELISA microplate, add:
   - 100 µl of the **Negative Control** to wells A1 and B1,
   - 100 µl of the **Positive Control** to wells C1 and D1,
   - 100 µl of the **pre-diluted samples** as prepared above.
3. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
4. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 3.**
5. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution 1X.** Avoid drying of the wells between washings.
6. Add 100 µl of the **Conjugate 1X** to each well.
7. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
8. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution 1X.** Avoid drying of the wells between washes.
9. Add 100 µl of the **Substrate Solution** to each well.
10. Cover the plate and incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
11. Add 100 µl of the **Stop Solution** to each well, in the same order as in step No. 9, to stop the reaction.
12. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean OD value of the Positive Control (OD_{PC}) is greater than 0.250.

$$\mathbf{OD_{PC} > 0.250}$$

✓ the ratio of the mean O.D. values of the Positive and Negative Controls (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%)

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

Samples presenting a S/P%:

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

Result	Status
S/P % ≤ 50 %	Negative
S/P % > 50 %	Positive

IDvet
Innovative Diagnostics

Certified management system

ID Screen®
Pasteurella multocida Duck Indirect

Indirect ELISA for the detection of antibodies directed against *P. multocida* in duck serum.

For *in vitro* use

July 2018
New protocol with ready-to-use Negative and Positive Controls

PMS-DUCK ver 0718 EN

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