General Information

This diagnostic kit is designed to detect specific antibodies against the non structural protein of the Foot and Mouth Disease virus (FMDV NSP) by competitive ELISA.

This method is suitable for serum or plasma from bovine, ovine, caprine, porcine and all susceptible species.

While both infection and vaccination elicit antibodies against structural antigens, only infected animals develop antibodies against the FMD virus non-structural protein (NSP). The FMD NSP ELISA can therefore be used as a DIVA test (Differentiation Infected and Vaccinated Animals) when highly purified vaccines are used.

The NSP protein being highly-conserved among the 7 FMD virus serotypes (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3), the test can be used to detect them all.

Description and Principle

Microwells are coated with the non structural protein of the Foot and Mouth Disease virus (FMDV NSP).

Samples to be tested and the controls are added to the microwells. Anti-NSP antibodies, if present, form an antigen-antibody complex which masks the virus epitopes.

An anti-NSP horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the remaining free epitopes, forming an antigen-conjugate-HRP-complex.

The excess conjugate is removed by washing, and the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested:

- In the absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.
- In the presence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Note: This kit does not contain any infectious material.

Kit Components

Reagents
Microplates coated with the FMDV NSP
Concentrated conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 18
Dilution Buffer 13
Wash Concentrate (20X)
Substrate Solution (TMB)
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. For detailed storage conditions of opened and/or diluted components, please refer to https://www.id-vet.com/fr/support/faq.
4. Wash and stop solutions can be used for the entire IDvet product range. Substrate solutions and dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µL, 30 µL, 50µL, 100µL and 500 µL.
2. Disposable tips.
3. 96-well microplate reader
4. Distilled or deionized water.
5. Manual or automatic wash system.

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. 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.
4. Do not expose the substrate solution to bright light nor to oxidating agents.
5. 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@innovative-diagnostics.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 **(20 X)** is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20 X)** 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 all reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or Vortex.

Serum and plasma: short incubation

1. Add:
   - 50 µL of the **Dilution buffer 18** to each well.
   - 30 µL of the **Positive Control** to wells A1 and B1.
   - 30 µL of the **Negative Control** to wells C1 and D1.
   - 30 µl of each sample to be tested in the remaining wells.
2. Cover the plate and incubate **2 hours ± 10 min** at 37°C (±3°C).
3. Empty the wells. Wash each well 5 times with at least 300 µL of the Wash Solution. Avoid drying of the wells between washes.

Serum and plasma: overnight incubation

1. Add :
   - 90 µl of the **Dilution Buffer 18** to each well.
   - 10 µl of the **Positive Control** to wells A1 and B1.
   - 10 µl of the **Negative Control** to wells C1 and D1.
   - 10 µl of each sample to be tested in the remaining wells.
2. Cover the plate and incubate 16-20 hours at 21°C (± 5°C).
3. Empty the wells. Wash each well 5 times with at least 300 µl of the Wash Solution. Avoid drying of the wells between washes.

For all protocols:

4. Prepare the **Conjugate 1X** by diluting the Concentrated Conjugate 10X to 1:10 in **Dilution Buffer 13.**
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 5 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 Negative Control O.D. (OD_{NC}) is greater than 0.7.

$$\mathbf{OD_{NC} > 0.700}$$

✓ The mean value of the Positive Control O.D. (OD_{PC}) is less than 30 % of the OD_{NC}.

$$\mathbf{OD_{PC}/OD_{NC} < 0.3}$$

Interpretation

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

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

Samples presenting S/N%:

- Less than or equal to 50 % are considered positive.
- Greater than 50 % are considered negative.

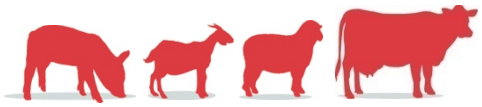
Result	Statut
S/N % ≤ 50 %	POSITIVE
S/N % > 50%	NEGATIVE

Note: The IDSoft™ data analysis program is available free-of-charge. Please contact, for more information, support.software@innovative-diagnostics.com.

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® FMD NSP Competition



Competitive ELISA for the detection of antibodies against the FMDV non structural protein (NSP) in serum or plasma from cattle, sheep, goats, swine and other susceptible species.

Usage *in vitro*

FMDNSPC ver 0914 EN

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