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.

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. Components bearing the same name (wash solution, dilution buffers) can be used for the entire IDvet product range.

Note: If necessary, IDvet has, at your disposal, additional volumes of reagents.

Materials required but not provided

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µL, 30 µL, 50µL, 100µL and 200 µL.
2. Disposable tips.
3. 96-well microplate reader
4. Distilled or deionized water.
5. Manual or automatic wash system.

Digitally signed by Cucu Vasile
Date: 2020.06.26 14:14:45 EEST
Reason: MoldSign Signature
Location: Moldova

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0,5 M) may be harmful if swallowed. It may cause sensitisation by skin contact **(R22-43)**. Avoid contact with skin **(S24-37)**.
4. Do not expose the substrate solution to bright light nor to oxidating agents.
5. All single-use material used for the assays should be decontaminated by immersion in freshly prepared 5% sodium hypochlorite for minimum 1 hour before elimination, or by autoclaving at 120°C.

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.

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. Incubate **2 hours ± 10 min** at 37°C (±3°C).
3. Empty the wells. Wash each well 5 times with approximately 300 µL of the Wash Solution. Avoid drying of the wells between washings.

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. Incubate 16-20 hours at 21°C (± 5°C).
3. Empty the wells. Wash each well 5 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.

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. Incubate **30 min ± 3 min** at 21°C (±5°C).
7. Empty the wells. Wash each well 5 times with approximately 300 µL of the **Wash Solution.** Avoid drying of the wells between washings**.**
8. Add 100 µL of the **Substrate Solution** to each well.
9. 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 order 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.

$OD_{NC} > 0.700$

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

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

Interpretation

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

$$S/N\ \% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

Samples presenting S/N%:

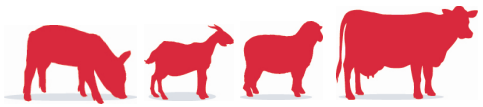
- Less than or equal to 50 % are considered positive.
- Greater than 50 % are considered negative.

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

IDvet
Innovative Diagnostics

Certified management system

ID Screen® FMD NSP Competition



Competitive ELISA for the detection of anti-FMDV non structural protein (NSP) antibodies in serum and plasma from bovine, ovine, caprine, porcine and all susceptible species.

Usage *in vitro*

FMDNSPC ver 0914 GB

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