PrioCHECK™ FMDV NS Ab Strip Kit

ELISA for *in vitro* detection of antibodies against non-structural protein of Foot and Mouth Disease Virus in serum of cattle, sheep, goats and pigs

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Introduction

Foot and Mouth Disease (FMD) is the most important economic threat to the livestock industry. The highly contagious disease affects all cloven-hoofed animals and is widespread over the world. The FMD viruses are classified into 7 distinct serotypes which makes diagnosis using conventional serological methods complex. To control outbreaks in the future emergency vaccination will be carried out. Vaccines consist of (partly) purified structural proteins of the FMD virus and therefore vaccinated animals only elicit antibodies directed against the structural proteins of the virus. However, after infection with FMDV, antibodies directed against the structural and the non-structural proteins are produced. Therefore an ELISA detecting antibodies against non-structural proteins of FMDV detects not only infected animals but also discriminates between infected and vaccinated animals.
The Applied Biosystems™ PrioCHECK™ FMDV NS Ab Strip Kit detects antibodies directed against the non-structural 3ABC protein of FMDV. The ELISA detects FMDV infected animals independent of the serotype that causes the infection and independent of the fact that the animal is vaccinated or not. The ELISA can be used to test serum samples of cattle, sheep, goats and pigs [1].

Test principle

The PrioCHECK™ FMDV NS Ab Strip Kit is a blocking ELISA. The Test Plates are coated with 3ABC specific monoclonal antibody (mAb), followed by incubation with antigen (3ABC protein). Consequently, Test Plates of the kit contain FMDV NS antigen captured by the coated mAb.
The test is performed by dispensing the test samples to the wells of a Test Plate. After incubation the plate is washed and the Conjugate is added. FMDV NS specific antibodies, directed against the non-structural proteins, that may be present in the test sample will bind to the 3ABC protein and will block the binding of the mAb-HRPO. After incubation, the plate is washed and the Chromogen (TMB) Substrate is dispensed. After incubation at room temperature (22±3°C) the color development is stopped. Color development measured optically at a wavelength of 450 nm shows the presence of antibodies directed against Foot and Mouth Disease Virus.
The PrioCHECK™ FMDV NS Ab Strip Kit is a single dilution test. The dilution of serum varies according to the protocol selected.

- Overnight serum incubation protocol: Serum samples are tested in a 1:5 dilution.
- Single-day serum incubation protocol: Serum samples are tested in a 1:2.6 dilution.

Kit components

5 plate strip kit for maximal 450 samples (in maximal 30 independent test runs). Store kit at 5±3°C until expiry date. See kit label for actual expiry date. The shelf life of diluted, opened or reconstituted components is noted below, when appropriate.

Component	Description
1: Test Plate	Five Test Plates are delivered in bags which contain a desiccant sachet.
2: Conjugate (30x)	30x concentrated, dilute before use. One vial contains 2.2 ml Conjugate. Diluted conjugate is not stable, prepare just before use.
3: Dilution Buffer (2x)	2x concentrated, dilute before use. One vial contains 60 mL Dilution Buffer. Shelf life of the dilution buffer working solution: 24 hours at 5±3°C.
4: Additive	Lyophilized. Reconstitute and dilute before use. Five vials, each contains 2.5 mL lyophilized Additive. Shelf life of reconstituted additive: until expiry date at –20°C.
5: Demineralized Water	Two vials, each contains 10 mL Demineralized Water.
6: Washing Fluid (200x)	200x concentrated, dilute before use. One vial contains 60 mL Washing Fluid. Shelf life of washing solution: 1 week at 22±3°C.
7: Positive Control	Ready-to-use. One vial contains 3.2 mL Positive Control.
8: Weak Positive Control	Ready-to-use. One vial contains 3.2 mL Weak Positive Control.
9: Negative Control	Ready-to-use. One vial contains 3.2 mL Negative Control.
10: Chromogen (TMB) Substrate	Ready-to-use. One vial contains 60 mL Chromogen (TMB) Substrate.
11: Stop Solution	Ready-to-use. One vial contains 60 mL Stop Solution.
Additional kit contents	• Package Insert • Aluminum bag • 1 lid to cover the strips during incubation

For Veterinary Use Only. For In Vitro Use Only.

Additional material required

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Use	Description
General	Laboratory equipment according to national safety regulations.
Analysis of results	Plate Reader. The reader has to have an appropriate filter set to read the plates at 450 nm.
Optional	Plate washer.

Test Procedure

Precautions

- National Safety Regulations must be strictly followed.
- The PrioCHECK™ FMDV NS Ab Strip Kit must be performed in laboratories suited for this purpose.
- Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Notes

To achieve optimal results with the PrioCHECK™ FMDV NS Ab Strip Kit, the following aspects must be considered:

- **The Test Procedure protocol must be strictly followed.**
- All reagents of the kit must be equilibrated to room temperature (22±3°C) before use.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Demineralized or water of equal quality must be used for the test.

Solutions to be made in advance

Dilution buffer working solution

Dilute Dilution Buffer (2x) (Component 3) 1:2 in demineralized water; e.g. for 2 strips prepare 4 mL (2.0 mL Dilution Buffer (2x) and 2.0 mL demineralized water). Can be stored at 5±3°C for up to 24 hours.

Additive

Equilibrate the vial to 22±3°C and reconstitute the lyophilized Additive (Component 4) with 2.5 mL Demineralized Water (Component 5). Can be stored at –20°C until expiry date.

Reconstitution of the lyophilized Additive should be performed as follows:

1. Equilibrate the vial to 22±3°C.
2. With the vial in an upright position, tap the vial gently against the worktop to ensure that the content is on the bottom of the vial.
3. Carefully open the vial.
4. Add the specified amount of Demineralized Water (Component 5).
5. Replace the stopper on the vial and allow the lyophilized material to dissolve.
6. Gently agitate the vial so that any remaining dry material will be dissolved.
7. Allow the material to stand at least for 15 minutes at 22±3°C before use.
8. Mix gently and intermittently (formation of foam should be avoided).

ELISA buffer

Dilute reconstituted additive 1:10 in dilution buffer working solution; e.g. for 2 strips prepare 4 mL (add 0.4 mL reconstituted additive to 3.6 mL dilution buffer). Unused ELISA buffer can be stored at 5±3°C for up to 24 hours.

Conjugate dilution

Dilute the Conjugate (30x) (Component 2) 1:30 in ELISA buffer; e.g. for 2 strips prepare 2.1 mL (add 70 µL Conjugate (30x) to 2.03 mL ELISA buffer).

Note: The diluted conjugate must be prepared just before use.

Washing solution

Dilute Washing Fluid (200x) (Component 6) 1:200 in demineralized water. The amount of Washing Fluid is sufficient to prepare a final volume of 12 liters washing solution. Stability of washing solution: 1 week stored at 22±3°C.

Remark: Commercial available ELISA washers can be used. If not available, washing of the plates can be done by dispensing at least 200 µL of washing solution to all wells of the plate. Subsequently, empty the plate and repeat as many times as prescribed. It is not necessary to soak the plate between washings. Tap the plate firmly after the last washing step.

Incubation with test serum

Perform the serum incubation using the overnight or single-day protocol. The required volumes of the test and control samples differ, depending on the protocol selected.

Overnight serum incubation protocol

1. Dispense 80 µL ELISA buffer to all wells of the Test Plate (Component 1).
2. Dispense 20 µL of Negative Control (Component 9) to wells A1 and B1.
3. Dispense 20 µL of Weak Positive Control (Component 8) to wells C1 and D1.
4. Dispense 20 µL of Positive Control (Component 7) to wells E1 and F1.
5. Dispense 20 µL of test samples to the remaining wells.
6. Cover the Test Plate with the enclosed lid.
7. Shake the Test Plate gently.
8. Incubate overnight (16–18 hours) at 22±3°C.

Single-day serum incubation protocol

1. Dispense 80 µL ELISA buffer to all wells of the Test Plate (Component 1).
2. Dispense 50 µL of Negative Control (Component 9) to wells A1 and B1.
3. Dispense 50 µL of Weak Positive Control (Component 8) to wells C1 and D1.
4. Dispense 50 µL of Positive Control (Component 7) to wells E1 and F1.
5. Dispense 50 µL of test samples to the remaining wells.
6. Cover the Test Plate with the enclosed lid.
7. Shake the Test Plate gently.
8. Incubate for 2 hours at 22±3°C.

Incubation with conjugate

Note: This procedure is performed on day two if the overnight serum incubation protocol is used.

1. Empty the Test Plate after the serum incubation period, then wash the plate 6 times with 200 to 300 µL of washing solution. Tap the plate firmly after the last washing step.
2. Dispense 100 µL of diluted conjugate to all wells.
3. Cover the Test Plate with the enclosed lid.
4. Incubate 60±5 minutes at 22±3°C.

Incubation with Chromogen (TMB) Substrate

1. Empty the Test Plate after the incubation period and wash the plate 6 times with 200 to 300 µL washing solution. Tap the plate firmly after the last washing step.
2. Dispense 100 µL of Chromogen (TMB) Substrate (Component 10) to all wells.
3. Incubate 20 minutes at 22±3°C.
4. Add 100 µL of Stop Solution (Component 11) to all wells.
5. Mix the content of the wells of the Test Plate prior to measuring.

Note: Start the addition of Stop Solution 20 minutes after the first well was filled with Chromogen (TMB) Substrate. Add the Stop Solution in the same order and at the same pace as the Chromogen (TMB) Substrate was dispensed.

Reading of the test and calculating the results

1. Measure the optical density (**OD**) of the wells at 450 nm within 15 minutes after color development has been stopped.
2. Calculate the mean OD_{450} value of wells A1 and B1 (Negative Control = OD_{450} **max**).
3. The percentage inhibition (**PI**) of the Controls and the test sera are calculated according to the formula below.

The OD_{450} values of all **samples** are expressed as Percentage Inhibition (**PI**) relative to the OD_{450} **max**.

$$PI = 100 - (OD_{450\ test\ sample} / OD_{450\ max}) \times 100$$

Result interpretation

Validation criteria

1. The OD_{450} **max** (mean OD_{450} of the Negative Control) must be >1.000.
2. The mean percentage inhibition of the Weak Positive Control must be >50%.
3. The mean percentage inhibition of the Positive Control must be >70%.

Not meeting any of these criteria is reason to discard the results of that specific Test Plate.

Note: If the OD_{450} of a **test sample** is higher than the OD_{450} **max**, the Percent Inhibition can be interpreted as 0%. If the mean OD_{450} of the Negative Control is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case warm the solution to 22±3°C or incubate up to 30 minutes. If the mean OD_{450} of the Negative Control is above 2.000 a shorter incubation period with the Chromogen (TMB) Substrate is recommended.

Interpretation of the percent inhibition

PI = <50%	Negative	Antibodies against the NS protein of FMDV are absent in the test sample.
PI = ⩾50%	Positive	Antibodies against the NS protein of FMDV are present in the test sample.

References

Sørensen KJ, Madsen KG, Madsen ES, Salt JS, Nqindi J, Mackay DKJ (1998) Differentiation of infection from vaccination in foot-and-mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Arch Virol* 143:1461–1476.

Customer and technical support

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- Worldwide contact telephone numbers
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- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision history of Pub. No. MAN0013927 (English)

Rev.	Date	Description
A.0	7 August 2019	• Converted the legacy document (PrioCHECK FMDV-NS 5 strip 7610770_v1.0_e.doc) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos. • Added a single-day serum incubation protocol. • Increased the volume of controls supplied with the kit from 1.4 mL to 3.2 mL. • Changed the product name from PrioCHECK® FMDV NS to PrioCHECK™ FMDV NS Ab Strip Kit.