# PrioCHECK™ FMDV Type A Ab Strip Kit

ELISA for *in vitro* detection of antibodies against Foot and Mouth Disease Virus serotype A in serum of cattle, sheep, goats and pigs

**– For Kazakhstan ONLY –**

**This document has been specifically developed in compliance with the requirements of the Ministry of Agriculture of the Republic of Kazakhstan.**

**For territories outside of Kazakhstan, see the standard documentation that is provided with the kit.**

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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 international standard test for antibody detection is the virus neutralization test (OIE Manual), but this test is laborious, and must be performed in a high security laboratory. Therefore, different ELISA's have been developed. However, most of these ELISA's make use of polyclonal sera, are single-species, and are time consuming, which hampers their suitability for mass screening and reproducibility. Therefore, the Applied Biosystems™ PrioCHECK™ FMDV Type A Ab Strip Kit is based on monoclonal antibodies (mAbs) for cattle, pigs, goats and sheep. The test is designed to obtain a minimum of reaction steps, allow mass screening, and is suitable for automation.

## Test principle

The PrioCHECK™ FMDV Type A Ab Strip Kit is a blocking ELISA and detects antibodies (Ab) against Foot and Mouth Disease Virus serotype A. The reaction between FMDV type A antigen and a specific monoclonal antibody (mAb) is blocked by specific antibodies that are present in the test sample.
A microtiter plate is coated with non-infectious FMDV type A antigen. Buffer and serum are added to the wells. The plate is incubated at room temperature (22±3°C). After washing, the mAb-HRPO conjugate is added to the plate and the plate is incubated at 22±3°C. The plate is washed and Chromogen (TMB) Substrate is added. After incubation, color development is stopped by adding Stop Solution and the optical density (OD) is measured at 450 nm.

## Kit components

5 plate kit for 450 samples. 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 containing 2.5 mL Conjugate. Diluted conjugate is not stable, prepare just before use. |
| 3: Dilution Buffer (5x) | 5x concentrated, dilute before use. One vial contains 60 mL Dilution Buffer. |
| 4: Horse Serum | Lyophilized. Reconstitute and dilute before use. Two vials, each vial contains 3.5 mL Horse Serum. Shelf life of reconstituted horse serum: until expiry date at –20°C. |
| 5: Demineralized Water | One vial 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: Negative Control | Ready-to-use. One vial contains 0.5 mL Negative Control. |
| 8: Weak Positive Control | Ready-to-use. One vial contains 0.5 mL Weak Positive Control. |
| 9: Positive Control | Ready-to-use. One vial contains 0.5 mL Positive 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<br>• 10 plate sealers |

## 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 Type A 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 Type A 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 (5x) (Component 3) 1:5 in demineralized water. (1 volume Dilution Buffer + 4 volumes demineralized water).

### Horse Serum

Equilibrate the vial to 22±3°C and reconstitute the Horse Serum (Component 4) with 3.5 mL Demineralized Water (Component 5). Reconstituted horse serum can be stored at –20°C until expiry date.

**Reconstitution of the lyophilized Horse Serum 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 horse serum 1:20 in dilution buffer working solution; e.g. for 1 plate prepare 22 mL (add 1.1 mL reconstituted horse serum to 20.9 mL dilution buffer working solution). ELISA buffer can be stored for up to 4 hours at 22±3°C.

### Conjugate dilution

Dilute Conjugate (30x) (Component 2) 1:30 in freshly prepared ELISA buffer; e.g. for 1 plate prepare 12 mL (add 400 µL Conjugate (30x) to 11.6 mL ELISA buffer).

**Note: The working dilution must be prepared just before use.**

### Washing solution

Dilute the 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 of 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 Test Plates can be done by dispensing at least 200 µL of washing solution to all wells of the Test Plate. Subsequently, empty the Test Plate and repeat as many times as prescribed. It is not necessary to soak the Test plate between washings. Tap the Test Plate firmly after the last washing step.

## Incubation of test serum

1. Dispense 80 µL of ELISA buffer to all wells of the Test Plate (Component 1).
2. Dispense 20 µL of Negative Control (Component 7) 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 9) to wells E1 and F1.
5. Dispense 20 µL of test samples to the remaining wells.
6. Seal and shake the Test Plate gently.
7. Incubate 60±5 minutes at 22±3°C.

## Incubation with conjugate

1. Empty the Test Plate after the incubation period and wash the plate 6 times with 200 µL to 300 µL washing solution. Tap the plate firmly after the last washing step.
2. Dispense 100 µL of the diluted conjugate to all wells.
3. Seal and shake the Test Plate gently.
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 µL 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 15 minutes at 22±3°C.
4. Add 100 µL of Stop Solution (Component 11).
5. Mix the content of the wells of the Test Plate.

**Note:** Start the addition of Stop Solution 15 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 of wells A1 and B1 (= $OD_{450}$ **max**).
3. The percentage inhibition (**PI**) of the Weak Positive Control, Positive Control and the test sera are calculated according to the formula below.

The $OD_{450}$ values of all **samples** are expressed as percentage inhibition 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** must be at least 1.000.
2. The mean percentage inhibition of Weak Positive Control must be >50%.
3. The mean percentage inhibition of 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 $OD_{450}$ **max** 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.

### Interpretation of the percent inhibition

| | | |
|---|---|---|
| PI <50% | Negative | FMDV type A antibodies are absent in the test serum. |
| PI ≥50% | Positive | FMDV type A antibodies are present in the test serum. |

**Note:** Samples with a PI value ≥50% are immune to Foot and Mouth Disease Virus serotype A.

## Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- 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.

## Limited product warranty

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**Revision history of Pub. No.** MAN0018879

| Rev. | Date | Description |
|---|---|---|
| A.0 | 4 April 2020 | • New document for use in Kazakhstan. Converted the legacy document (PrioCHECK FMDV A 5 plt 7610850 v1.2_e.doc) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.<br>• Added "For Kazakhstan ONLY" statement to the document heading and a note, regarding immune samples, to "Interpretation of the percent inhibition" on page 2. |