Test Validation

- The mean of the **Negative Controls** (NC) must produce an optical density ≥ 0.40 and ≤ 1.60.
- The %CV for the **Negative Controls** must be ≤ 15%.
- The mean of the **Positive Controls** must have an inhibition of 50-70%.

Interpreting the Results

- Test samples having < 40% inhibition are negative.
- Test samples having ≥ 40% inhibition are positive.

Precautions

Kit components should be handled and disposed of as potentially hazardous. Do not eat, drink, or smoke where serum samples and kit reagents are handled. Do not pipette by mouth. Some reagents may be harmful if ingested. If ingested, seek medical attention. Do not use expired or contaminated reagents, or reagents from other kits or serials. Do not mix reagents from different serials of this same product.

Component B, Positive Control, contains sodium azide as a preservative.

Component C, Negative Control, contains sodium azide as a preservative.

Component D, 100X Antibody-Peroxidase Conjugate, contains ProClin 300, methylisothiazolinone, bromonitrodioxane, and thimerosal as preservatives.

Component E, Conjugate Diluting Buffer, contains ProClin 300, methylisothiazolinone, and bromonitrodioxane as preservatives.

Component F, Serum Diluting Buffer, contains sodium azide as a preservative.

Version 170323

Assay Limitations: This assay may give false positive or false negative results. Test results should be interpreted in the context of all available clinical, historical and epidemiological information relevant to the animals under test. Further confirmatory testing may be required to validate results.

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VLN/PCN 332/5FM0.20

FOOT AND MOUTH DISEASE VIRUS ANTIBODY TEST KIT

Assay instructions for catalog numbers: 5FM0.20-2 and 5FM0.20-5.

General Description

This Foot and Mouth Disease Virus (FMDV) Antibody Test Kit is a competitive, enzyme-linked, immunosorbent assay (cELISA) for the detection of antibodies specific for FMDV in bovine, porcine and ovine serum samples. It is intended to provide results which will give guidance about the presence of FMDV infection. The principle of the test is as follows: Sample serum antibodies to FMDV inhibit the binding of a horseradish peroxidase (HRP)-labeled monoclonal antibody to the FMDV antigen coated on the plastic wells. Binding, or lack of binding, of the HRP-labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent color product development. Strong color development indicates little or no blockage of HRP-labeled monoclonal antibody binding and therefore the absence of antibodies to FMDV in the sample serum. Weak or no color development due to inhibition of the monoclonal antibody binding to the antigen on the solid phase indicates the presence of FMDV antibodies in the sample serum.

Kit Contents

	Component	5FM0.20-2	5FM0.20-5
A	Antigen-Coated Plates	2 plates	5 plates
B	Positive Control	4 ml	4 ml
C	Negative Control	4 ml	4 ml
D	100X Antibody-Peroxidase Conjugate	0.3 ml	0.5 ml
E	Conjugate Diluting Buffer	30 ml	60 ml
F	Serum Diluting Buffer	11 ml	25 ml
G	10X Wash Solution Concentrate	120 ml	2 × 120 ml
H	Substrate Solution	30 ml	60 ml
I	Stop Solution	30 ml	60 ml
	Instructions for Use		

Materials Required But Not Included in the Test Kit

Single and multichannel adjustable-volume pipettors and disposable plastic tips, non-antigen-coated transfer plate(s), ELISA microplate absorbance spectrophotometer with 450 nm filter, deionized or distilled water, Parafilm® or equivalent, paper towels, graduated cylinder, timer, multichannel pipettor reservoirs, wash bottle, manual multichannel washing device or automatic plate washer

Storage and Stability

Store all reagents at 2-8°C. **Do not freeze.** Reagents will remain stable until the expiration date when stored as instructed. **Do not use test kit past the expiration date printed on the box.**

Preparation

a. **Warm reagents:** Bring the serum samples, reagents and plates to room temperature (23 ± 2°C) prior to starting the test.

b. **Prepare samples:** Test serum samples must be diluted 1/2 in Serum Diluting Buffer (F) for use in this test. Add at least 35 µl but no more then 120 µl Serum Diluting Buffer (F) to the appropriate wells of a transfer plate according to your setup record, leaving empty wells for subsequent addition of controls. Add an equal volume of sample to each well containing the serum diluting buffer and cycle the pipettor at least three times to mix. Change tips between samples.

c. **Prepare plates:** Remove the plate(s) from the foil pouch(es) (A). If applicable: Return any unused strips to the pouch and securely seal it. Place strips to be used in the frame and number the top of each strip to maintain orientation. Always mark the strips in case they dislodge from the frame during washing.

d. **Prepare conjugate:** Prepare 1X Antibody-Peroxidase Conjugate by diluting one part of the 100X Antibody-Peroxidase Conjugate (D) with 99 parts of Conjugate Diluting Buffer (E). Example: For 96 wells, mix 60 µl of 100X Antibody-Peroxidase Conjugate (D) with 5.940 ml of Conjugate Diluting Buffer (E) to yield 6 ml of 1X Antibody-Peroxidase Conjugate. Fifty microliters (50 µl) are needed per well. Allow extra quantity for reservoirs, tubing, pipetting, etc.

e. **Prepare wash solution:** Prepare 1X Wash Solution by diluting 1 part of the 10X Wash Solution Concentrate (G) with 9 parts of deionized or distilled water. At least 1.2 ml are needed per well. Allow extra quantity for reservoirs, tubing, pipetting, etc.

Test Procedure

1. **Load controls and serum samples:** Positive and Negative controls are provided READY-TO-USE. Load Positive Control (B) in duplicate and Negative Control (C) in triplicate, regardless of the number of serum samples to be tested. Add the controls to the empty transfer plate wells from Preparation step b. When whole plates are used, it is best to put the controls in wells on different areas of the plate. The controls must be loaded every time the assay is performed and on each plate if multiple plates are used. Using a multichannel pipettor set at 50 µl, load controls and diluted serum samples into the Antigen-Coated Plate (A). Serum samples and controls should be loaded in the Antigen-Coated Plate (A) as quickly as possible. Change tips between rows or columns. Tap the side of the Antigen-Coated Plate (A) several times to make sure the samples coat the bottom of the wells. Use care not to spill samples from well to well. If running the assay in a hood, cover the plate with Parafilm® or equivalent to prevent evaporation and incubate the plate 90 minutes at room temperature (23 ± 2°C).

2. **Wash wells:** After the 90-minute incubation, wash the plate 3 times.

   If an automatic washer is used, place the plate on the washing apparatus and wash plate 3 times, filling the wells each time with at least 200 µl of 1X Wash Solution.

   If manual washing is used, dump well contents and remove remaining sera and controls by sharply striking the inverted plate 4 times on a clean paper towel, striking a clean area each time. Immediately fill each well with at least 200 µl of 1X Wash Solution using a multichannel filling device or a wash bottle. Empty the wash solution from the plate and strike the inverted plate sharply on a clean paper towel as above. Fill and empty the plate by the same method 2 additional times for a total of 3 washes.

3. **Add conjugate:** Add 50 µl of diluted (1X) Antibody-Peroxidase Conjugate to each well. Tap the side of the loaded assay plate several times to make sure the conjugate coats the bottom of the wells. Incubate the plate for 30 minutes at room temperature (23 ± 2°C).

4. **Wash wells:** After the 30-minute incubation, wash the plate 3 times as in Step 2.

5. **Add substrate solution:** Add 50 µl of Substrate Solution (H) to each well. Tap the side of the loaded assay plate several times to make sure the substrate coats the bottom of the wells. Incubate the plate for 20 minutes at room temperature (23 ± 2°C). Avoid leaving the plate in direct sunlight. *Do not empty wells.*

6. **Add stop solution:** Add 50 µl of Stop Solution (I) to each well. Tap the side of the loaded assay plate several times to mix the Substrate Solution and the Stop Solution. *Do not empty wells.*

7. **Read and record the test results:** Immediately after adding the Stop Solution, the plate should be read on a microplate absorbance spectrophotometer. Set the optical density (OD) reading wavelength to 450 nm and read plate(s). Some readers require an empty well on the plate for blanking. In this case, no reagents should be added to this well.

8. Return all remaining kit reagents to 2-8°C for storage.

Calculations:

% Inhibition (%I) = 100 [1-(Sample OD ÷ NC Mean OD)]

%CV = 100 (Standard Deviation/NC Mean OD)

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