HRPro® FMDV NSP AB ELISA

CAT.NO. EM -FMD-01

GENERAL DESCRIPTION

Foot and mouth disease (FMD) is the most contagious disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals. There are seven serotypes of FMD virus, namely, O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1.

The demonstration of specific antibodies to non-structural proteins (NSP) are useful in providing evidence of previous or current viral replication in the host, irrespective of vaccination status. NSPs, unlike structural proteins, are highly conserved and therefore are not serotype specific and as a consequence, the detection of these antibodies is not serotype restricted. The OIE had prescribed serological test for NSP antibodies by EITB, AGP of VIAA and ELISA It can be used in surveillance program of FMD vaccinated or non-vaccinated area.

HRPro® FMDV-NSP is based on innovative recombinant NSP to detect antibodies against nonstructural protein of FMDV. The test kit detects NSP specific antibodies independent of serotype and independent of vaccination. The ELISA can be used to serum from cattle, pigs, goats and sheep without additional reagents.

HRPro® FMDV-NSP is a blocking ELISA based on recombinant NSP, 3AB immobilized on ELISA plates and Peroxidase labeled monoclonal antibodies.

The test is performed by dispensing test samples to the wells of NSP antigen coated plate. After incubation and washing, the conjugate is added. FMDV NSP antibodies if it contains in the test samples will bind to the NSP and will block the binding epitope of HRPO Anti-FMDV NSP conjugated Mab. After incubation and washing, the TMB substrate is dispensed. If binding of the HRPO conjugate is blocked by NSP antibodies in test samples, the unbound conjugate will be washed away and less or no color will be developed. If the HRPO conjugate has bound to antigen color will be developed, it indicate the NSP antibodies are absent in test samples.

The result is obtained by comparing the absorbance (OD), which develops in wells containing the samples with the OD from the wells containing the negative control (S/N ratio).

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KIT COMPONENTS

	Reagents	480 tests
①	FMDV 3AB Coated Plate	5 plates
②	10X Washing Buffer	240㎖ X 1
③	Dilution Buffer	60㎖ X 1
④	HRPO Anti-FMDV NSP Conjugate	70㎖ X 1
⑤	Positive Control, PC	2.0㎖ X 1
⑥	Negative Control, NC	2.0㎖ X 1
⑦	TMB Substrate	70㎖ X 1
⑧	Stop Solution	40㎖ X 1
⑨	Sealing Film	5 sheets
⑩	Instruction Manual	1 copy

PREPARATION

1. All reagents must be allowed to come to room temperature (20~ 25℃) before use. Mix reagents by gentle swirling.
2. 1X washing buffer preparation
   1) Shake 10X Washing Buffer(②) gently.
   2) Dilute 1 part of 10X Washing Buffer(②) with 9 parts of deionized water. The diluted 1X washing buffer is stable for 2 weeks at room temperature (20~ 25℃).
3. Sample preparation
   1) Serum samples (bovine, swine, sheep and goats, etc.) may be used in this assay.
   2) Use fresh samples for the best result. Serum samples can be stored at 2~8℃ for less than 3 days or –20℃ for a longer period. Do not freeze and thaw serum samples repeatedly. Sera with hemolysis or bacterial contamination are not suitable for the analysis!
   3) Visible solid materials in serum samples should be separated by centrifugation.
4. TMB Substrate (⑦) should be warmed up for 30 minutes at room temperature (20~ 25℃) before use (10㎖/plate). If stored at low temperature, the color development may be poor.

TEST PROCEDURE

1. Remove the Antigen Coated Plate (①) from protective foil pouch.
2. Dispense 80☒ of Dilution buffer(③) into each well of the FMDV 3AB Coated Plate(①).
3. Add 20☒ of samples, PC(⑤) and NC(⑥) into appropriate wells of plate containing dilution buffer. Final dilution factor is 1:5. Use care not to spill samples from well to well.
4. Seal the plate and incubate for 60 minutes at room temperature (25± 1.0℃).
5. Wash each well 3 times with 1X washing buffer (300☒ per well). And remove contents of well at each washing steps. After final washing, hit plate to the paper towel briefly to remove solution.
6. Dispense 100☒ of HRPO Anti-FMDV NSP conjugate(④) to each well.

7. Seal the plate and incubate for 60 minutes at room temperature (25± 1.0℃).
8. Wash each well as step 5.
9. Dispense 100☒ of TMB substrate solution to each well.
10. Seal the plate and incubate for 15 minutes at room temperature(25± 1.0℃). Check the density of color development by naked eyes.
11. Add 50☒ of Stop Solution (⑧) to each well of the plate. Shake the test plate shortly (5~10 sec). Be careful not to spill.
12. Measure and record the A (450nm) for samples and controls immediately.
13. Validate and calculate the results.

Plate template example (1-well Test)

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	13	21	29	37	45	53	61	69	77	85
B	NC	NC	14	22	30	38	46	54	62	70	78	86
C	1	7	15	23	31	39	47	55	63	71	79	87
D	2	8	16	24	32	40	48	56	64	72	80	88
E	3	9	17	25	33	41	49	57	65	73	81	89
F	4	10	18	26	34	42	50	58	66	74	82	90
G	5	11	19	27	35	43	51	59	67	75	83	91
H	6	12	20	28	36	44	52	60	68	76	84	92

RESULT INTERPRETATION

1. Validate if the mean OD of the NC is higher than 0.600 and the mean OD of the PC is lower than 0.300. If these criteria are not met, the test are invalid and the samples must be retested.
2. Calculate the sample to negative ratio (SN) by following the formula

$$SN = \frac{\text{Sample OD}}{\text{NC mean OD}}$$

3. Result interpretation
   1) Test samples having ≤0.60 S/N are positive.
   2) Test samples having > 0.60 S/N are negative.

S/N value	Interpretation
S/N ≤ 0.60	Positive
S/N > 0.60	Negative

4. Example of result calculation and interpretation
   1) ODs of PC : 0.085, 0.091
      Mean OD = (0.085 + 0.091) / 2 = 0.088 (valid)
   2) ODs of NC :1.121, 1.201
      Mean OD = (1.121 + 1.201) / 2 = 1.161 (valid)
   3) OD of Sample : 0.431
      S/N value of the sample = (0.431 / 1.161) = 0.371
   4) Result interpretation: Positive

PRECAUTIONS

1. All reagents must be allowed to come to room temperature (20~ 25℃) before use. Mix reagents by gentle swirling. After use, return to 2~8℃.
2. Read this instruction manual thoroughly and follow all steps strictly for successfuluse of the product.
3. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
4. Do not use expired or contaminated reagents.
5. Do not use reagents from other kits or lots.
6. Do not mix reagents from different lots of this same product.
7. Do not expose the reagents to excessive heat or direct light during storage and incubation.
8. Incomplete washing adversely may affect the result and precision of the assay.
9. Avoid microbial contamination of the reagents.
10. Avoid contamination of the TMB Substrate(⑦) with the HRPO Anti-FMDV NSP Conjugate (④).
11. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
12. Do not eat, drink, smoke or apply cosmetics where kit reagents are handled. Do not pipette by mouth.
13. Pipette tips must be changed after each pipetting step. Use a clean disposable pipette tip for all steps.
14. Use care not to spill samples from well to well.
15. Deionized water or equal must be used to prepare the washing buffer.
16. Unused strips should be stored in the sealed foil pouch at 2~8℃. Re-sealed strips are recommended to use within one week.
17. For veterinary use only.

STORAGE AND STABILITY

Store all reagents at 2~8℃. Do not freeze. Reagents remain stable until the expiration date when stored as instructed.

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