1.0 Intended Use and Composition

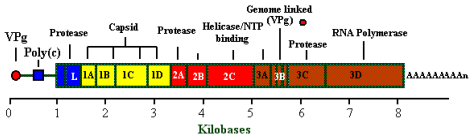
Arsh Biotech Foot and Mouth Disease (FMD) $3AB_3$ DIVA kit is an immunoassay to detect antibodies against the $3AB_3$ non-structural protein of FMD virus in bovine serum or plasma. This kit is available in 2 formats: For 192 tests (AB-$3AB_3$-002) and for 384 tests (AB-$3AB_3$-004). The kit components are as under:

Description	Part No.	AB $3AB_3$-002 192 Tests	AB $3AB_3$-004 384 Tests
ELISA Solid Plate (uncoated)	AEP01	2	4
FMD $3AB_3$ Protein	AAB1	1	2
Positive Control	APC01	1	2
Negative Control	ANC01	1	2
100X AB-Enzyme Conjugate	AEC01	150µl	250µl
Enzyme Substrate	AES01	12 ml	25 ml
Coating Buffer	ACB01	15 ml	25 ml
50X Wash Buffer	AWB50	20 ml	40 ml
Sample/Conjugate Diluent	ASD01	50ml	100ml
Stop Solution	AHS01	15 ml	25 ml

2.0 General Information

Foot and Mouth Disease is a highly infectious viral disease that affects cloven-hoofed animals including domestic and wild bovids. The causative agent is Foot and Mouth Disease Virus (FMDV), a member of genus Aphthovirus in the family Picornaviridae. FMD affects millions of farm animals in the world that are of great economic importance. Global impact of FMD outbreak has been estimated tens of billions of dollars. In India alone, direct losses due to FMD are estimated at Rs 20,000 crores.

The FMDV virus contains a single stranded positive sense RNA. The P1 region of the genome encodes structural proteins and the P2 and P3 encode regulatory or non-structural proteins. The RNA is translated as a single long polyprotein, followed by post-translational proteolytic cleavages to generate four structural proteins (VP1-4) and many non-structural proteins (L, 2A, 2B, 2C, 3A, 3B, 3C and 3D).



FMDV genome and function.

Vaccination based control programmes for FMD virus are being implemented in many countries. The identification of infected animals among the vaccinated animals is important for appropriate implementation of these FMDV control programmes. Antibodies to capsid proteins are induced by both vaccination and infection, however antibodies against NSPs are elicited only during FMD infection. Antibodies against the $3AB_3$ NSP is used as a common marker for the detection of FMD virus.

Arsh Biotech FMD $3AB_3$ DIVA (differentiation of infected from vaccinated animals) kit is designed to detect antibodies elicited against $3AB_3$ NSP in bovines.

3.0 Principle of the Test

Sero-conversion in response to the $3AB_3$ NSP is observed after 8-10 days of FMDV infection, whereas if the animal is not exposed to FMDV but is vaccinated with inactivated purified polyvalent FMD vaccine, no anti-NSP immune response is elicited. This differential induction of anti-NSP antibody is exploited in FMD $3AB_3$ DIVA ELISA to discriminate between infected and vaccinated animals. In this DIVA test, reactivity of anti-$3AB_3$ antibodies present in the serum of an infected animal is assessed against purified $3AB_3$ NSP in an indirect ELISA format. A sample producing signal value more than the recommended cut-off ratio compared to the positive controls is qualitatively diagnosed as positive for FMD infection. (Mohapatra *et al.,* 2011 J.Virol.Methods Vol 177:184-92)

4.0 Storage and Stability

Kit components are stable at ambient temperature. Upon receipt, store at 4°C. If unopened, the kit is stable until 6 months from the date of manufacture. Expiration date is printed on the kit label.

5.0 Preparation of Reagents

5.1 To be diluted: Store as indicated on labels.

Component	Preparation Instructions
100X AB-Enzyme Conjugate Part No. AEC01 (Store at 4°C)	Dilute the contents of the supplied vial to 1X with Sample/Conjugate Diluent. For 1 plate, dilute 60µl of conjugate in 5.94ml Diluent.
50X Wash Buffer Part No. AWB50 (Store at 4°C)	Dilute the entire volume of bottle with deionized water to make 1X solution and store at 4°C. For 1 plate, dilute 7.5 ml wash buffer in 367.5ml water.

Dilute reagents as necessary. Do not keep diluted reagents for more than 24 hours.

5.2 To be reconstituted: Store as indicated on labels.

Component	Preparation Instructions
Positive Control Part No. APC01	Reconstitute each vial in 2.4ml of sample diluent. Store at 4°C and do not use beyond 24 hours
Negative Control Part No. ANC01	Reconstitute each vial in 2.4ml of sample diluent. Store at 4°C and do not use beyond 24 hours
FMD $3AB_3$ Protein Part No. AAB1 (Coating Antigen)	Reconstitute each vial in 11 ml of coating buffer Store at 4°C and do not use beyond 24 hours

5.3 Ready for use: Store as indicated on labels.

Components	Store at
Coating Buffer Part No. ACB01	4°C
Enzyme Substrate Part No. AES01	4°C
Stop Solution Part No. AHS01	4°C

6.0 Materials required but not supplied

Description	Cat No.
Microplate reader to read wells at 450-620 nm wavelength.	ABMR01
Automatic microplate washer to wash wells.	ABMW01
Pipettes that dispense 20-100µl and 100-1000 µl.	ABMP100 ABMP1000
Low protein binding sterile 1 ml tubes or deep well plates for diluting samples.	ABOE01
Sterile tube of 15 ml capacity for diluting AB-Enzyme Conjugate.	ABOF15
Graduated cylinder of 1L capacity to dilute wash buffer concentrate.	ABOC1000
Store bottle to store diluted wash buffer.	ABOSB5
Sterile deionized water to dilute concentrated wash buffer.	ABOW500

7.0 Precautions and Safety Instructions

Standards, Sample Diluent, Conjugate Diluent and Enzyme-conjugate contain bromonitrodioxane (BND). Stop solution contains a proprietary acid solution. Follow good laboratory practices and avoid ingestion or contact of any reagent with eye, skin and mucous membrane. All the reagents may be disposed of down a drain with copious amount of water. MSDS for TMB and BND can be requested from AB or obtained at www.arshbiotech.com

8.0 Assay Procedure

8.1 Coating

Coat 96-well plate with the diluted $3AB_3$ protein (prepared in Section 5.2) at 50µl per well.

↓

Tap the plate gently from all sides to ensure that there is no bubble or uncovered space and incubate at 37°C for 60 Minutes. Alternatively, coat and incubate overnight at 4°C.

↓

Remove the plate from the incubator. If the coating was performed overnight at 4°C then keep the plate at 37°C for 15 minutes and proceed further.

↓

Wash three times continuously with 250µl of wash buffer per well.

8.2 Sample Preparation and Loading

Dilute the test sample at 1:100 in sample diluent. For example, if using test samples in duplicate, dilute 2.5µl of test sample in 247.5µl of diluent.

↓

Transfer 100µl of diluted test samples, supplied positive and negative controls (as prepared in Section 5.2) in the respective wells of coated ELISA plate. For background control add only 100µl of sample diluent buffer.

↓

Incubate at 37°C for 60 minutes.

↓

Wash three times with 250µl of wash buffer with three minutes soak time between washes.

8.3 Detection with AB-Enzyme Conjugate

Dispense 1X AB-Enzyme conjugate at 50 µl per well and incubate at 37°C for 60 minutes.

↓

Wash three times with 250 µl of wash buffer with five minutes soak time between washes.

↓

Transfer Enzyme substrate at 50µl per well and incubate at 37°C for 15 minutes.

8.4 Stop Reaction and Measurement

Stop the reaction by adding Stop Solution at 50µl per well and measure absorbance within the next five minutes.

↓

Read absorbance by using microplate reader at 450 nm (with 620 nm reference).

9.0 Interpretation

The final results for each test serum sample need to be expressed as Percentage Positive (PP) value.

$$\text{PP Value} = \frac{\text{Test sample mean OD}}{\text{Positive control mean OD}} \times 100$$

The results should be interpreted based on the following cut-off value:

Results	Positive	Negative
	PP Value ≥40%	PP Value <40%

For example, OD values of:
Background Control = Mean 0.02 (0.01, 0.03)
Negative Control = Mean 0.03 (0.02, 0.04)
Positive Control = Mean 1.60 (1.50, 1.70)
Sample 1=0.2; PP value < 40; **Result**: Negative
Sample 2=1.0; PP value > 40; **Result**: Positive

10.0 Validity of the Test

For the assay to be valid:

1. Positive control OD must not be less than 0.8
2. Negative control OD must not be more than 20PP.
3. Background control OD must be less than 10PP.
4. The difference in OD of duplicate wells of positive control must not be more than 20 percent.

11.0 Sample Plate Set-Up

Recommended Plate Layout for 45 Test Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	BC	BC	6	6	14	14	22	22	30	30	38	38
B	NC	NC	7	7	15	15	23	23	31	31	39	39
C	PC	PC	8	8	16	16	24	24	32	32	40	40
D	TS1	TS1	9	9	17	17	25	25	33	33	41	41
E	2	2	10	10	18	18	26	26	34	34	42	42
F	3	3	11	11	19	19	27	27	35	35	43	43
G	4	4	12	12	20	20	28	28	36	36	44	44
H	5	5	13	13	21	21	29	29	37	37	45	45

Abbreviations: TS= Test Sample, PC= Positive Control, NC= Negative Control, BC= Background Control.

N.B Tests are recommended to be performed in duplicates, both for samples and controls.

12.0 Common Troubleshooting Tips

Problem	Possible Cause	Solution
No signal or weak signal	Exclusion of key reagents	Check that all reagents have been added in the correct order.
	Old 1X Enzyme Conjugate	Prepare 1X AB-Enzyme Conjugate fresh and do not store it more than 24 hours.
	Enzyme inhibitor present	Contaminants such as Sodium azide inhibit enzyme reactions. Ensure a clean working area and use clean tips.
	Incorrect assay temperature (too cold)	Use recommended incubation temperature. Bring Enzyme Substrate to room temperature before use.
High Background	Poor Washing	Ensure all wells are filled with wash buffer and are being aspirated completely. Use an automated plate washer if available.
	Enzyme Conjugate too high	Use recommended dilutions of reagents.
	Contaminating enzymes present in diluent	Test diluent with substrate alone to check for contaminating enzyme activity.
Black/Brown Precipitate	Delay in reading the microplate	Ensure that the absorbance measurements are made within 5mins of adding the Stop Solution
	Highly Positive Sample	Run the test at greater sample dilution such as 1:100 or 1:200
	Conjugate not washed properly	Ensure all wells are washed properly with the recommended hold time between washes.
Uneven colour development	Incomplete washing of wells	Ensure all wells are filled evenly with wash buffer and are being aspirated completely.

Complete troubleshooting guide available at www.arshbiotech.com

13.0 FMD Product Basket

Arsh Biotech offers a portfolio of FMD assays which could be used for differentiation of infected from vaccinated animals, and products which could be used for sero-monitoring of the protecting antibody levels in vaccinated animals.

ELISAs for Non Structural Components of FMDV

Product Description	Species	Cat No.
FMD 3AB$_3$ DIVA ELISA Kit	Bovine	AB-3AB3-002
	Pig	AB-3AB3-102
	Goat/Sheep	AB-3AB3-202
FMD 3ABC DIVA ELISA Kit	Bovine	AB-3ABC-002
	Pig	AB-3ABC-102
	Goat/Sheep	AB-3ABC-202
FMD 2ABC IgG Indirect-ELISA Kit	Bovine	AB-2ABC-002
	Pig	AB-2ABC-102
	Goat/Sheep	AB-2ABC-202
FMD 3D IgG Indirect-ELISA Kit	Bovine	AB-3D-002
	Pig	AB-3D-102
	Goat/Sheep	AB-3D-202
FMD 3ABC Antibody Rapid Test	Bovine	AB-3ABC-R-002
	Pig	AB-3ABC-R-102
	Goat/Sheep	AB-3ABC-R-202
FMD 3ABC IgG Competitive ELISA Kit	All Species	AB-3ABC-C-002

ELISAs for Structural Components of FMDV

Product Description	Species	Cat No.
FMD O LPBE Elisa Kit	All Species	AB-LPBE-O-002
FMD A LPBE Elisa Kit	All Species	AB-LPBE-A-002
FMD Asia 1 LPBE Elisa Kit	All Species	AB-LPBE-X-002
FMD O, A, Asia 1 Combo LPBE Elisa Kit	All Species	AB-LPBE-AOX-002
FMD O VP-1 IgG ELISA Kit	Bovine	AB-VP1-O-002
	Pig	AB-VP1-O-102
	Goat/Sheep	AB-VP1-O-202
FMD A VP-1 IgG ELISA Kit	Bovine	AB-VP1-A-002
	Pig	AB-VP1-A-102
	Goat/Sheep	AB-VP1-A-202
FMD Asia 1 VP-1 IgG ELISA Kit	Bovine	AB-VP1-X-002
	Pig	AB-VP1-X-102
	Goat/Sheep	AB-VP1-X-202
FMD SAT-1 VP-1 IgG ELISA Kit	Bovine	AB-VP1-S1-002
	Pig	AB-VP1-S1-102
	Goat/Sheep	AB-VP1-S1-202
FMD SAT-2 VP-1 IgG ELISA Kit	Bovine	AB-VP1-S2-002
	Pig	AB-VP1-S2-102
	Goat/Sheep	AB-VP1-S2-202

For complete product listing please visit us at www.arshbiotech.com

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FMD 3AB$_3$ DIVA KIT

To Differentiate Infected from Vaccinated Animals (Bovine/Cow)

INSTRUCTION MANUAL
FOR ELISA KIT #AB-3AB$_3$-002 (192 Tests)
AND #AB-3AB$_3$-004 (384 Tests)

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Version 1.05