## 1.0 Intended Use and Composition

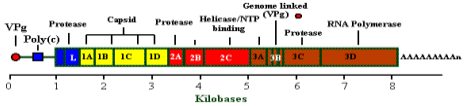
Arsh Biotech Foot and Mouth Disease (FMD) Single dilution Liquid Phase Blocking ELISA (SdLPBE) kit is an immunoassay for quantification of protective antibody levels against FMD Serotype O, A and Asia 1 in animals following vaccination. One kit is sufficient for testing 960 samples (AB-SdLPBE-045). The kit components are as under:

| Description | Part No. | Quantity |
|---|---|---|
| ELISA Solid Plate | AEP01 | 45 |
| Serotype Specific Anti-FMDV Coating Antibody | ACO01-18 | 5 |
| | ACA01-18 | 5 |
| | ACX01-18 | 5 |
| Serotype Specific Internal Serum Controls | ASCO1 to O8 | 8 (1 each) |
| | ASCA1 to A8 | 8 (1 each) |
| | ASCX1 to X8 | 8 (1 each) |
| Serotype Specific Antigens | AAO01-18 | 5 |
| | AAA01-18 | 5 |
| | AAX01-18 | 5 |
| Sample Diluent | ASD01-200 | 5 |
| Blocking Buffer | ABB01-18 | 1 |
| Serotype Specific Anti-FMDV Tracing Antibody | ATO01-18 | 5 |
| | ATA01-18 | 5 |
| | ATX01-18 | 5 |
| 100X Conjugate Antibody | ACC-2.7 | 1 |
| Conjugate Diluent | ACD01-18 | 15 |
| Enzyme Substrate | ASF01-18 | 15 |

## 2.0 General Information

Foot and Mouth Disease is a highly infectious viral disease that affects cloven-hoofed animals. 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 about 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 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), encoded by 1A, 1B, 1C,1D and




*FMDV genome and function*



many non-structural proteins (L, 2A, 2B, 2C, 3A, 3B, 3C and 3D). Antibodies to capsid proteins are induced by both vaccination and infection. Vaccination based control programmes for FMD virus are being implemented in many countries including India. For effective implementation of vaccination in animals following vaccination, antibody titres need to be quantified by Liquid Phase Blocking ELISA.

Arsh Biotech FMD Single dilution Liquid Phase Blocking ELISA (SdLPBE) kit is designed to estimate antibody titre against structural protein of FMDV serotypes O, A and Asia 1.

## 3.0 Principle of the Test

The principle of the LPBE assay is liquid-phase blocking of FMDV antigen by specific antibodies in the sera. In this, test serum sample is mixed with equal volume of a constant dose of viral inactivated antigen in a liquid medium and allowed to react. The antigen-antibody reaction is carried out in a suspension (or liquid medium), and the antigen is blocked by the homologous antibodies, if present, in the test serum for subsequent detection by guinea pig serum. The antigen molecules which are not completely blocked by the antibodies in the test serum are trapped to the wells of the ELISA plates with the pre-coated type-specific rabbit antibodies. Subsequently, the bound antigen is traced by sero-type specific guinea pig serum and anti-guinea pig-HRPO conjugate and substrate reaction is followed in a standard ELISA procedure. OD of antigen controls are used for normalization of data in terms of percentage inhibition and titre is calculated as the $\log_{10}$ of the inverse dilution at which percentage inhibition is 50%. Generally protective antibody level is expressed as $\log_{10}$ titre and titre of 1.8 is accepted as the protective level. In SdLPBE only one dilution of serum sample is tested and titre is extrapolated from the known internal serum controls.

***(Sharma, G.K. et al., 2015, Biologicals (43) 158-164)***

## 4.0 Storage and Stability

Kit components are stable at ambient temperature. Upon receipt, store components at -20°C and +4°C, as prescribed on the box. If unopened, the kit is stable until 6 months from the date of supply.

## 5.0 Preparation of Reagents

To be reconstituted:

| Component | Preparation Instructions |
|---|---|
| Serotype Specific Anti-FMDV Coating Antibody. | Reconstitute each vial with 18ml Double Distilled Water. |



| Component | Preparation Instructions |
|---|---|
| Serotype Specific Internal Serum Controls | Reconstitute each vial with 100 µl Double Distilled Water. Upon Reconstitution: Short Term Storage at +4°C. Long Term Storage at -20°C |
| Sample Diluent | Reconstitute one sachet in 200 ml Double Distilled Water. |
| Serotype Specific Antigens | Reconstitute each vial with 18ml Sample Diluent. |
| Blocking Buffer | Reconstitute each vial with 10 ml 1X Wash Buffer (WB). |
| Serotype specific anti-FMDV tracing antibody | Reconstitute each vial with 18ml 1X Wash Buffer (WB). |
| Conjugate/Secondary Antibody | Reconstitute each vial of Conjugate Diluent with 18ml 1X Wash Buffer and add 180µl of 100X Conjugate Antibody. |
| Enzyme Substrate | Reconstitute each vial in dark with 18ml Double Distilled Water and add 10µl Perhydrol® per 18ml to prepare 1X Working Substrate Solution. |

It is recommended that all components are reconstituted freshly before use.

## 6.0 Materials required but not supplied

| Description | Cat No. |
|---|---|
| Stop solution (1M $H_2SO_4$). | ABSS |
| Wash Buffer (with 0.1% Tween20) | AWBT |
| Microplate reader to read wells at 492-620 nm. | ABMR01 |
| Microplate washer to wash wells. | ABMW01 |
| Pipettes that dispense 20-100µl and 100-1000 µl. | ABMP100 ABMP1000 |
| Low protein binding deep well plates. | ABDW |
| Sterile tube of 50 ml capacity. | ABFT50 |
| Graduated cylinder of 1L capacity. | ABGC1000 |
| Double Distilled Water | ABDDW |
| Perhydrol® (from Merck®) containing 30% Hydrogen Peroxide. | 1.07210 (Merck®) |

*Alternatively, for preparing 1L of1X wash buffer, mix 0.39g $NaH_2PO_4.2H_2O$, 1.33g $Na_2HPO_4.2H_2O$, 29.33g NaCl, 1mL Tween-20 in water and set pH at 7.2.*

## 7.0 Precautions and Safety Instructions

Enzyme Substrate contains OPD (Ortho-phenylene-diamine). Follow good laboratory practices and avoid ingestion or contact of any reagent with eye, skin and mucous membrane. All the reagents should be disposed properly in hazardous bags. MSDS for OPD can be requested from AB or can be obtained at www.arshbiotech.com.



## 8.0 Assay Procedure

### 8.1 Coating

**Coat** 96-well plate with the reconstituted antibody (described in Section 5.0) 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, incubate the plate at 4°Covernight.

⬇

**Remove** the plate from the incubator.
If the coating was performed overnight at 4°C, keep the plate at 37°C for 15 minutes before proceeding to wash the plate.

### 8.2 Liquid Phase Blocking using Test Sample

**Dilute** the test sample at 1:32 in Sample Diluent (6µl test sample in 192 µl of Sample Diluent).

⬇

**Transfer** 50 µl of diluted serum into three low binding deep well plates (for O, A and Asia1) as per the recommended format in Section 11.0.

⬇

**Add** serotype specific reconstituted antigen into diluted serum at 50 µl per well.

### 8.3 Liquid Phase Blocking using Internal Controls

**Prepare** Internal serum controls for each serotype in Two-fold dilutions from 1:16 to 1:64 using the above deep well plates in the recommended format.

(Add 6.25 µl of Internal serum control prepared in Section 5.0 to 93.75 µl of Sample Diluent in Column 1 of the plate. Then, add 50 µl of Sample Diluent to Column 2 and Column 3, and transfer 50 µl from Column 1 to the next Column sequentially till Column 3. Finally discard 50 µl from Column 3)

⬇

**Add** serotype specific reconstituted antigen into diluted Internal Serum Controls at 50 µl per well.

⬇

**Add** 50 µl of same Antigen in antigen control wells followed by adding 50 µl Sample Diluent.

⬇

**Incubate** deep well plate containing antigen-antibody mixture at 37°C for 60 minutes. Alternatively, incubate overnight at 4°C.

### 8.4 Transfer of Ag-Ab Mixtures to Coated Plate

**Wash** the Coated Plates prepared in Section 8.1 three times with 250µl of wash buffer with three minutes soak time, between washes.

⬇

**Transfer** 50µl of Ag-Ab Mixtures from the deep well plates containing the test samples, internal serum controls and antigen controls in the respective wells of coated Elisa plates. For background control add 50µl of blocking buffer prepared in Section 5.0.

⬇

**Incubate** at 37°C for 60 minutes.

⬇

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

### 8.5 Tracing Serum and Conjugate Addition

**Dispense** reconstituted serotype specific tracing antibody at 50 µl per well and incubate at 37°C for 60 minutes.

⬇

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

⬇

**Dispense** reconstituted 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 three minutes soak time between washes.

### 8.6 Colour Development and Measurement

**Dispense** 50 µl per well of freshly prepared Substrate Solution as described in Section 5.0 and incubate at 37°C for 10-15 minutes.

⬇

**Stop** the reaction by adding 1M $H_2SO_4$at 50µl per well and measure absorbance.

⬇

**Read** absorbance by using microplate reader at 492 nm (with 620 nm reference).



## 9.0 Validity of the Test

**The assay to be valid if:**

1. Antigen control OD for all three serotypes is greater than 0.8.
2. Background control OD is less than 0.15.

## 10.0 Interpretation

The final results for each test serum sample can be expressed as Percentage Inhibition (PI) value as under:

$$\text{PI Value} = \left(1 - \frac{\text{Test sample OD}}{\text{Antigen control mean OD}}\right) \times 100$$

Titre of the test samples is expressed as the reciprocal of the serum dilution which gives a PI value of 50%. Higher the PI value higher the antibody titre.

For doing the calculations of the titres for the test samples, please log on to www.arshbiotech.com and download the MS excel macros using secure code provided along with the kit. Do not use MS excel file of another batch of kit to do the calculations, as it will lead to incorrect interpretation.

## 11.0 Recommended Plate Layout

**Recommended Plate Layout for 64 Test Samples**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Serum Control 1 | | | AGC | TS1 | TS2 | TS3 | TS4 | TS5 | TS6 | TS7 | TS8 |
| B | Serum Control 2 | | | AGC | TS9 | TS10 | TS11 | TS12 | TS13 | TS14 | TS15 | TS16 |
| C | Serum Control 3 | | | AGC | TS17 | TS18 | TS19 | TS20 | TS21 | TS22 | TS23 | TS24 |
| D | Serum Control 4 | | | AGC | TS25 | TS26 | TS27 | TS28 | TS29 | TS30 | TS31 | TS32 |
| E | Serum Control 5 | | | AGC | TS33 | TS34 | TS35 | TS36 | TS37 | TS38 | TS39 | TS40 |
| F | Serum Control 6 | | | AGC | TS41 | TS42 | TS43 | TS44 | TS45 | TS46 | TS47 | TS48 |
| G | Serum Control 7 | | | BGC | TS49 | TS50 | TS51 | TS52 | TS53 | TS54 | TS55 | TS56 |
| H | Serum Control 8 | | | BGC | TS57 | TS58 | TS59 | TS60 | TS61 | TS62 | TS63 | TS64 |
| | 1:16 | 1:32 | 1:64 | | | | | | | | | |

**Abbreviations**: TS=Test Sample, AGC= Antigen Control and BGC= Background Control.

**Technical Support available**

**Toll Free at 1800-3000-8822**



## 12.0 Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| **No signal or weak signal** | Exclusion of key reagents | Check that all reagents were prepared as prescribed in section 5.0 and have been added in correct order. |
| | Cross serotype contamination | Ensure all the reagents like Coating antibody, Tracing antibody and Antigen have been dispensed properly. Handle different serotypes separately to avoid chances of cross serotype contamination. |
| | Incorrect assay temperature (too cold) or incubation time | Use recommended incubation temperature. Ensure Enzyme Substrate is at room temperature before use. Substrate incubation time may be increased to 20 mins. |
| **High Background or Uneven colour development** | Substrate Exposed | Protect the substrate from light during reconstitution. Ensure the reconstituted substrate is colourless before adding to the microplate. |
| | Poor Washing | Ensure all wells are filled with wash buffer and are being aspirated completely. Ensure all wells are washed properly with the recommended hold time between washes. |
| | Mistake in preparation of reagents | Prepare reagents by reconstituting in appropriate volume of diluents as recommended in section 5.0. |
| **Signal Overflow** | Incorrect assay temperature (too warm) or incubation time. | Use recommended incubation temperature. Substrate incubation time may be decreased. |

Complete troubleshooting guide available at www.arshbiotech.com

## 13.0 FMD Product Basket

*Arsh Biotech provides a wide range of diagnostic assays for FMDV structural and non-structural proteins.*

| Product Description | Species | Cat No. |
|---|---|---|
| **FMD SdLPBE (O, A , Asia1)** | All Species | AB-SdLPBE-045 |
| **FMD LPBE (O, A , Asia1)** | All Species | AB-LPBE-045 |
| **FMD 3ABC DIVA ELISA Kit** | Bovine | AB-3ABC-002 |
| | Pig | AB-3ABC-102 |
| | Goat/Sheep | AB-3ABC-202 |
| **FMD 3AB$_3$ DIVA ELISA Kit** | Bovine | AB-3AB3-002 |
| | Pig | AB-3AB3-102 |
| | Goat/Sheep | AB-3AB3-202 |

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



# FMD Single Dilution Liquid Phase Blocking ELISA KIT

To determine antibody levels against FMD Serotypes O, A and Asia 1

**INSTRUCTION MANUAL**

**FOR ELISA KIT #AB-SdLPBE-045 (960 Samples)**

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