Foot and Mouth Disease Virus (FMDV) -NSP Antibody (3ABC) GENLISA™ ELISA (for cattle, sheep, goat and pig)

REF : KAD1102

Ver 1.1

RUO

Qualitative testing for Foot and Mouth Disease Virus (FMDV) -NSP Antibody (3ABC) in samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

Foot-and-mouth disease virus (FMDV) Non-structural protein Antibody 3ABC ELISA Test Kit is suitable for test serum of cattle, sheep, goats and pigs, it can distinguish between immunized animals and wild-infected animals.

Intended Use:

The Foot and Mouth Disease Virus (FMDV) -NSP Antibody (3ABC) GENLISA™ ELISA is used as an analytical tool for qualitative laboratory screening of presence or absence of Foot and Mouth Disease Virus (FMDV) -NSP Antibody (3ABC) in the serum.

Principle:

The method employs indirect ELISA technique. Foot and Mouth Disease Virus (FMDV) is pre-coated onto microwells. Samples and controls are pipetted into microwells and Foot and Mouth Disease Virus (FMDV) -NSP Antibody (3ABC) Antibodies ELISA present in the sample are bound by the antigens. Then Anti-FMD antibody: Enzyme Conjugate is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Foot and Mouth Disease Virus (FMDV) -NSP Antibody (3ABC) Antibodies ELISA in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Foot and Mouth Disease Virus Antigen Coated Microtiter Plate (8 x 12 wells) – 2 no
2. Negative control – 2 ml
3. Positive control – 1 ml
4. Then Anti-FMD antibody: Enzyme Conjugate – 2x11 ml
5. Sample Diluent – 100 ml
6. (20X) Wash Buffer – 4x25 ml
7. TMB Substrate – 2x12 ml
8. Stop Solution –2x12 ml
9. Instruction Manual

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Specimen Collection

Sample Preparation: Take animal whole blood, get serum by using regular method, the serum should bright and no hemolysis

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 25 ml of 20 X Wash Buffer in 975 ml of DI water.

Procedural Notes:

1. Return all reagents into room temperature before use, put the reagents at room temperature for at least 1 hour. Shake it evenly before use, and store back to 2-8℃ after usage.
2. Do not mix use reagents from different kits and different lot no., prevent the reagents been polluted when using.
3. TMB Substrate and stop solution may have irritation to skin and eyes, be careful to use.
4. Do not expose Substrate to strong light and avoid contact with the oxidant.
5. H5 Antigen coated plates should be sealed and moisture-proof. Put back unused Micro-Well plate into dry foil bag and sealed at 2-8℃.
6. All wastes should be treated well to avoid pollution before discarding.
7. Strict compliance with the operating instructions can get the best results. Pipetting operation, timing, and washing of the whole process must be precise.
8. H5 Antigen Coated plates is disposable, do not repeat use.

Assay procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. All steps must be performed at Room Temperature.

2. Pipette **100 ul of Positive Control and Negative Controls** into the respective wells.

3. Add **80 ul of sample diluent then add 20 ul of sample** into the respective wells.

4. Cover the plate with a sealer and incubate at 37°C fo**r 90 minutes at 37°C.**

5. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.

6. Add **100ul Anti-FMD antibody: Enzyme Conjugate** to the wells.

7. Incubate at 22°C for 60 minutes.

8. Repeat the wash step 5.

9. Add **100 ul** of **TMB Substrate** solution into each well.

10. Cover the plate with sealer & Incubate at 37°C for 15 minutes in dark

11. Add **50 ul** of **Stop Solution** to each well.

12. Read the absorbance at 450nm in a microplate reader within 10 minutes.

Calculation of the results:

Determine the Mean Absorbance for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

$$\text{Inhibition Rate} = 1 - \left[\frac{\text{Mean Absorbance of Sample}}{\text{Mean Absorbance of Negative control}} \right]$$

Validity of the test:

The test is valid if the following condition is met,
Negative control (N) Absorbance value > 0.6
Inhibition rate of Positive Control > 50%

Interpretation of the results:
Positive Sample: Inhibition rate > 50%;
Negative Sample: Inhibition rate $\leq$ 50%

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from cattle, sheep, goat and pig fluids or organs used in the preparation of this kit. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

1. In any case GLP should be applied with all general and individual regulations to the use of this kit.

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