LT Biotech

Operating instruction
«LTELISA FMD» indirect ELISA kit for detection FMD virus antibodies

REF LT-E-FMD-01

Version: 002-2017-09

General Description:

This test kit is designed for detection of G isotype antibodies (IgG) for Foot and Mouth disease (FMD) virus in samples of animal serum, and can differentiate FMDV-infected from vaccinated animals.

Serum antibodies bind to non-nstructural protein 3ABC attached to the plastic wells of the polystyrene plate. Antibodies binding is detected with horseradish peroxidase (HRP)-labeled recombinant protein G. Attachment of HRP-labeled conjugate is visualized with enzyme substrate (chromogen TMB) and subsequent blue color product development. Intensive color development indicates the presence of IgG to FMD virus in the serum.sample. Very weak or no color development indicates the absence of antibodies to FMD virus in the serum sample.

This kit allows assaying of 184 samples, without control samples.

Animal species: cattle, pigs, sheep, goats, and wildlife species.

Kit Contents:

Component	Quantity
Antigen-coated plate	2 plates
Conjugate immunoenzyme (x100)	1 vial, 0.3 ml
Wash solution concentrate (x30)	2 vials, 15.0 ml
Stop reagent	1 vial, 12.0 ml
Serum dilution buffer	2 vials, 12.0 ml
Substrate Solution (TMB)	1 vial, 21.0 ml
Positive control (C+)	1 vial, 0.1 ml
Negative control (C–)	1 vial, 0.1 ml
Sticky film	2 pieces
Operating instruction	1 piece

Preparation of reagents:

1. Warm up reagents at room temperature (18–22) ºC during 30 minutes.
2. Prepare (1X) Wash solution. Intensively shake Wash solution concentrate (15ml) and dilute with **435.0 ml** of distilled or deionized water. If Wash solution concentrate contains sediment dissolve crystals by heating at (35–37) ºC. Keep (1X) Wash solution in clean vial tightly closed at temperature (2–8) ºC no longer than 5 days.
3. Prepare conjugate solution. Conjugate solution (1X) has to be prepared directly before use. Dilute one part of Conjugate (x100) with 99 parts of Wash solution (1X) in a clean vial. Mix well avoiding foaming.
   For example:
   For one strip add 1.0 ml of Wash solution (1X) to clean bottle, and then add 0.01 ml of Conjugate (x100). Mix well by pipetting.
   For plate add 10.0 ml of Wash solution (1X) to clean bottle, and then add 0.1 ml of Conjugate (x100). Mix well by pipetting.
4. Prepare serum specimens. Samples containing aggregates and/or precipitate should be clarified by centrifugation for 10 minutes at 2500-3000 RPM. Serum samples may be stored at (2-6) °C no longer than three days. It is allowed to store the serum samples at - 20°C (or lower) for three months and at - 70 °C for two years. Do not freeze and thaw sera specimens twice. Sera with sodium azide, hemolysis, hyperlipidemia or bacterial contamination are not suitable for the analysis!

Test Procedure:

1. Remove protective foil from antigen-coated plate and add **0.090 ml** of Serum dilution buffer into all wells of the plate.
2. Add **0.010 ml** of serum samples into wells of the plate leaving free of the first 4 wells (A1, B1, C1, D1) for Positive and Negative controls.
3. Add **0.010 ml** of the Positive control (C+) into wells A1, B1, and **0.010 ml** of the Negative control (C–) into wells C1, D1. Carefully repipette the mixture in wells. Take care not to spill samples from well to well. During the pipetting solution in wells may be changed in color. Stick the plate by enclosed film and incubate at temperature **37.0ºC for one hour**.
4. Rinse the plates 4 times with **0.35 ml** of a 1X Wash solution using microplate washing machine: aspirate the well contents completely, fill up the wells at each rinse and aspirate completely. Make sure that no fluid remains on the strips and stripholder after the last aspiration. If necessary, dry the plate by tapping on absorbent paper.
5. Add **0.1 ml** of 1X Conjugate solution (see above) into each well. Stick the plate by enclosed film and incubate at temperature **37.0ºC for 30 minutes**.
6. Rinse the plates 6 times with **0.35 ml** of 1X Wash solution following washing procedure described in point #4.
7. Add **0.1 ml** of Substrate Solution (TMB) into each well. Incubate plate at **20–25^{0}C for 30 minutes**. Avoid leaving the plate in direct sunlight.
8. Stop colour reaction by adding **0,05 ml** of Stop Solution to each well.
9. Measure the optical density (OD) of the controls and samples at 450/620 nm using a microplate reader. To prevent the fluctuation in OD values measure OD within 3 minutes after reaction was stopped.

Result interpretation:

1. Calculate the mean OD-values for each control (mean OD Negative Control and mean OD Positive Control).
2. Calculate the percent positivity (PP) values for negative controls and samples, using the formula:

$$PP = \frac{Mean\ OD\ sample\ or\ Negative\ control}{Mean\ OD\ Positive\ control} \times 100$$

For example:

$$PP = \frac{0.765}{2.832} \times 100\% = 27\%$$

3. Analysis is considered correct, if mean value of OD Positive Control is greater than 1.0, mean value of PP Negative Control is less than 8.

4. Results of the analysis for examined samples are considered **Positive** if PP of the samples are equal or greater than 8%**.** Results of the analysis for examined samples are considered **Negative if PP of the samples are less than 8%.**

5. **8%** - the constant value, which is determined by the manufacturer and may be changed in different LOT numbers.

Storage and Stability:

Store all reagents at 2-8^{0}C. **Do not freeze**. Reagent will remain stable until the expiration date when stored as recommended. Do not use test kit beyond the expiration date printed on the label.

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