LT Biotech

Operating instruction

«LTELISA RVF IgM» for detection antibodies to Rift Valley fever in animal serum

REF **LT-E-RVF-IgM-01**

Version: 001-2017-05

General Description:

This test kit **«RVF LTelisa Ab IgM»** is one step immunoassay designed for detection of M isotype antibodies (IgM) to Rift Valley fever virus (RVFV) in animal serum.

Wells of polystyrene microwell strips are coated with RVFV recombinant antigen. When investigated animal serum and conjugate are placed into wells, RVFV-specific IgM bind both to recombinants antigens on the solid phase and to horseradish peroxidase (HRP)-labeled antigens in the conjugate forming antigen-antibody complexes. Addition of enzyme substrate (chromogen TMB) results in development of blue colored product in the case HRP-labeled conjugate is present in the well. Strong color development indicates the presence of IgM to RVFV in the sample sera. Very weak or no color development indicates the absence of IgM to RVFV in the sample sera.

Kit allows assaying of 92 samples, without control samples.

Animal species: ruminants.

Kit Contents:

Component	Quantity
Antigen-coated plate	1 plate
Conjugate immunoenzyme (x100)	1 vial, 0.15 ml
Wash solution concentrate (x30)	1 vial, 15.0 ml
Serum dilution buffer	1 vial, 12.0 ml
Stop reagent	1 vial, 6.0 ml
Substrate Solution (TMB)	1 vial, 11.0 ml
Positive control (C+)	1 vial, 0.1 ml
Negative control (C–)	1 vial, 0.1 ml
Sticky film	1 piece
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 at temperature (2–8) ºC no longer than 5 days in clean vial tightly closed.
3. Prepare conjugate solution. Conjugate solution (1X) has to be prepared before use. Dilute one part of Conjugate (x100) with 99 parts of **Serum dilution buffer** in a clean vial. Mix well avoiding foaming.
   For example:
   For one strip add 1.0 ml of **Serum dilution buffe**r to clean bottle, and then add 0.01 ml of Conjugate (x100). Mix well by pipetting.
   For plate add 10.0 ml of **Serum dilution buffer** 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, haemolysis, hyperlipidemia or bacterial contamination are not suitable for the analysis!

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Test Procedure:

1. Remove protective foil from antigen-coated plate.
2. Add **0.09 ml** conjugate solution (see above) into each well of the plate.
3. Add **0.01 ml** of serum samples into wells of the plate leaving free first 4 wells (A1, B1, C1, D1) for Positive and Negative controls.
4. Add **0.01 ml** of the Positive control (C+) into wells A1, B1, and **0.01 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 90 minutes**.
5. Rinse the plates 6 times with 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.
6. Add **0.1 ml** of Substrate Solution (TMB) into each well. Incubate plate at **20–25^{0}C for 15 minutes**. Avoid leaving the plate in direct sunlight.
8. Stop colour reaction by adding of **0,05 ml** Stop Solution to each well.
9. Measure the optical density (OD) of the controls and samples at 450/620 nm using a microplate photometer. To prevent the fluctuation in OD values measure OD within 3 minutes after reaction was stopped.

Interpreting the Results:

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 controls and samples, using the formula:

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

For example:

$$PP = \frac{2.255}{2.92} \times 100\% = 77.2\%$$

3. Analysis is considered correct, if mean value of OD Positive Control are greater than 0.8, mean value of PP Negative Control are less than 8.
4. Results of the analysis for investigated samples are considered **Positive** if PP samples are equal or greater than 20%**.** Results of the analysis for investigated samples are considered **Negative if PP samples are less than 20%.**
5. **20%** - the constant value is established by the manufacturer and may be changed in various 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 past the expiration date printed on the label.

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