General information

This diagnostic kit is designed to detect antibodies directed against the nucleoprotein (NP) of the Rift Valley Fever Virus (RVFV) in serum or plasma by competitive ELISA.

The detection of anti-nucleoprotein antibodies indicates exposure to the virus by natural infection or by vaccination.

This test can be used with samples from ruminants, horses, cats, dogs, humans (for research use only) or from any other susceptible species.

Description and principle

Wells are coated with a recombinant RVFV nucleoprotein (NP).

Samples to be tested and the controls are added to the wells. Anti-nucleoprotein antibodies, if present, form an antibody-antigen complex which masks the nucleoprotein epitopes.

After washing, an anti-nucleoprotein antibody-peroxidase (HRP) conjugate is added to the wells. It fixes to the remaining free nucleoprotein epitopes, forming an antigen-conjugate-HRP complex.

After washing in order to eliminate the excess conjugate, the Substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested:

- in the absence of antibodies, a blue coloration appears which becomes yellow after addition of the Stop solution.
- in the presence of antibodies, no coloration appears.

The microplate is read at 450 nm.

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Kit components

Reagents*
Coated Microplates
Concentrated conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 19
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

** Quantities supplied are indicated on the kit label.*

1. The Conjugate, Controls and Substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. For detailed storage conditions of opened and/or diluted components, please refer to www.innovative-diagnostics.com/storage-conditions
4. Wash and Stop solutions can be used for the entire IDvet product range. Substrate solutions and Dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel micropipettes capable of delivering volumes of 100 µL and 500 µL.
2. Disposable tips.
3. 96-well microplate
4. Distilled or deionized water.
5. Manual or automatic microplate wash system.
6. ELISA microplate reader.

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitisation by skin contact. Avoid contact with skin and eyes. Use protective lab coat, one-way gloves and safety glasses. The Stop solution (0,5 M acid) may be harmful if swallowed.
3. Do not expose the Substrate solution to bright light nor to oxidizing agents.
4. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

Please refer to the Material Safety Data Sheet, available upon request at info@innovative-diagnostics.com for more detailed information.

Sample preparation

In order to avoid differences in incubation times between samples, it is possible to prepare a 96-well microplate containing the test and control samples, before transferring them into an ELISA microplate using a multi-channel pipette.

Wash Solution preparation

If necessary, bring the **Wash Concentrate (20X)** to room temperature (21°C ± 5°C) and mix thoroughly to ensure that it is completely solubilised.

Prepare the **Wash Solution (1X)** by diluting the **Wash Concentrate (20 X)** to 1:20 in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the "IDvet Washing Guide", available upon request.

Testing procedure

Allow all the reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or vortexing.

1. In the ELISA microplate, add:
   - 50 µL of **Dilution Buffer 19** to each well.
   - 50 µL of the **Positive Control** to wells A1 and B1.
   - 50 µL of the **Negative Control** to wells C1 and D1.
   - 50 µL of **each sample to be tested** to the remaining wells.
2. Cover the plate and incubate **60 minutes** ± **6 min** at 37°C (± 2°C).
3. Empty the wells. Wash each well 3 times with at least 300 µL of the **Wash Solution**. Avoid drying of the wells between washes.
4. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 19.**
5. Add 100 µL of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 minutes** ± **3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with at least 300 µL of the **Wash Solution**. Avoid drying of the wells between washes.
8. Add 100 µL of the **Substrate Solution** to each well.
9. Cover the plate and incubate **15 minutes** ± **2 min** at 21°C (± 5°C) in the dark.
10. Add 100 µL of the **Stop Solution** to each well in the same order as in step No. 8 to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean O.D. value of the Negative Control (OD_{NC}) is greater than 0.7.

$$\mathbf{OD_{NC} > 0.7}$$

✓ the mean O.D. value of the Positive Control (OD_{PC}) is less than 30% of the OD_{NC}.

$$\mathbf{OD_{PC} / OD_{NC} < 0.3}$$

Interpretation

For each sample, calculate the competition percentage (S/N%).

$$\mathbf{S/N\% = \frac{OD_{sample}}{OD_{NC}} x100}$$

Samples presenting S/N%:

- Less than or equal to 40% are considered positive.
- Greater than 40% and less than or equal to 50% are considered doubtful.
- Greater than 50% are considered negative.

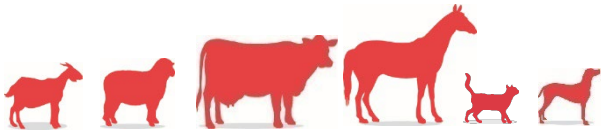
Result	Status
S/N% ≤ 40%	POSITIVE
40% < S/N% ≤ 50%	DOUBTFUL
S/N% > 50%	NEGATIVE

Note: The IDSoft™ data analysis program is available free-of-charge. For more information, please contact support.software@innovative-diagnostics.com.

This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested).

Certified management system

ID Screen®
Rift Valley Fever Competition Multi-species



Competitive ELISA for the detection of antibodies against the RVFV nucleoprotein in serum or plasma from ruminants, horses, cats, dogs
humans (for research use only)
or any other susceptible species

For *in vitro* use

January 2024:
Human samples testing now available for Research Use Only (RUO), without modifications in the instructions for use (protocol and cut-off values). Please refer to publications and peer-reviewed references for more information.

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