Version 28-04/25

User's Manual

Rift valley fever IgM capture ELISA kit

REF **DEIA-CL018**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This diagnostic kit is designed to detect IgM antibodies directed against the Rift Valley Fever (RVF) nucleoprotein (NP) in bovine, ovine and caprine serum or plasma.

This kit is an IgM Antibody Capture ELISA (MAC), a specific method for the detection IgM antibodies. The presence of IgM antibodies in the serum sample indicates recent infection.

General Description

Wells are coated with anti-bovine-ovine-caprine IgM polyclonal antibodies.

Samples and controls are added in duplicate to adjacent even and odd-numbered wells. The IgM antibodies present in the sample bind to the coated anti-bovine-ovine-caprine IgM polyclonal antibodies.

Plates are washed and a RVF nucleoprotein is added to the even-numbered columns only (No RVF nucleoprotein is added to the odd-numbered columns). The RVF nucleoprotein fixes to the anti-RVF IgM antibodies present in the sample and captured on the plate.

After washing and elimination of excess RVF nucleoprotein, an anti-RVF NP monoclonal antibody conjugated to HRP is added. It fixes to the nucleoprotein previously captured on RVF IgM antibodies.

After washing and elimination of 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 presence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.

- in the absence of antibodies, no coloration appears.

The microplate is read at 450nm.

Reagents And Materials Provided

1. Microplates coated with anti-bovine IgM antibody
2. Freeze-dried RVF Nucleoprotein (10X)
3. Reconstitution Buffer
4. Anti-RVF NP HRP conjugate (10X)
5. Positive Control
6. Negative Control
7. Dilution Buffer 11
8. Dilution Buffer 13
9. Dilution Buffer 18
10. Wash Concentrate (20X)
11. Substrate Solution
12. Stop Solution (0.5 M)

*Quantities supplied are indicated on the kit label.

1. The conjugate, the controls and the substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. Components bearing the same name (wash solution, dilution buffers) can be used for the entire IDvet product range.

Materials Required But Not Supplied

1. Mono or multi-channel micropipettors capable of delivering volumes of 10 µl, 100 µl, and 200 µl.
2. Disposable tips.
3. 96-well microplate reader.
4. Distilled or deionized water.
5. Manual or automatic wash system.

Storage

Store the kit at 4°C upon receipt. For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

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

Reagent Preparation

Wash Solution Preparation

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

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

RVF Nucleoprotein 10X Preparation

Add 1ml of Reconstitution Buffer to the Freeze-dried RVF Nucleoprotein vial. Wait 1-2 minutes and mix well in order to homogenize the solution.

The reconstituted RVF Nucleoprotein may be stored:

- 4 weeks at 5°C (± 3°C)

- for long-term storage, aliquot and freeze (< - 18°C). Each aliquot may undergo 3 freezing-thawing cycles without any loss of activity, and may be stored for 12 months.

Assay Procedure

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

Attention: Each sample must be deposited twice (adjacently in even and odd-numbered wells). See Figure 1 below.

1. Add:

− 40 µl of Dilution Buffer 18 to each well.

− 10 µl of the Negative Controlto wells A1, B1 and A2, B2.

− 10 µl of the Positive Control to wells C1, D1 and C2, D2.

− 10 µl of each sample to be tested in duplicate to the remaining wells(each sample must be deposited twice in adjacent even and odd numbered wells – please refer to Figure 1 below).

2. Incubate 1h±5 min at 37°C (± 3°C).
3. Preparethe RVF Nucleoprotein 1X by diluting the RVF Nucleoprotein 10X to 1/10 in Dilution Buffer 13.

	D13	RVF -NP	D13	RVF -NP	D13	RVF -NP	D13	RVF -NP	D13	RVF -NP	D13	RVF -NP
	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	NC	NC	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	PC	PC	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	PC	PC	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

Figure 1: Plate map. Samples are deposited in duplicate in adjacent even and odd-numbered wells.

NC=negative control. PC=positive control. S=sample. D=dilution buffer.

RFV- NP= Rift Valley Fever nucleoprotein.

4. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.
5. Add 50 µl of the RVF nucleoprotein 1X to the even-numbered columns only. Add 50 µl of Dilution Buffer 13 to the odd-numbered columns.
6. Incubate 1h± 5 min at 37°C (± 3°C).
7. Prepare the Conjugate 1Xby diluting the Conjugate 10X to 1/10 in Dilution Buffer 11.
8. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.
9. Add 50 µl of the Conjugate 1X to each well.
10. Incubate1h± 5 min at 37°C (± 3°C).
11. Wash each well 3 times with approximately 300 µl of the Wash Solution. Avoid drying of the wells between washings.
12. Add 100 µl of the Substrate Solutionto each well.
13. Incubate 15 min± 2 min at 21°C (± 5°C) in the dark.
14. Add 100 µl of the Stop Solution to each well in order to stop the reaction.
15. Read and record the OD at 450 nm.

Calculation

Calculate the net OD results (to be used for the validation and interpretation steps):

net OD = OD even well – **OD** odd well

The test is validated if:

1. the mean value of the net Positive Control OD (net OD PC) is greater than 0.350.

net OD PC > **0.350**

2. the ratio of the mean values of the net Positive and Negative Control ODs (net ODPC and net OD NC) is greater than 3. In this formula, use the absolute value of the net OD NC.

net OD PC / |**net OD**NC | > **3**

Interpretation Of Results

For each sample, calculate the S/P percentage (S/P%):

S/P % = net OD sample/net OD PC x 100

Samples presenting a S/P percentage (S/P %):

- less than or equal to 40% are considered negative.

- between 40% and 50% are considered as doubtful

- greater than or equal to 50% are considered positive.

Result	Status
S/P % ≤ 40%	NEGATIVE
40% < S/P% < 50%	DOUBTFUL
S/P % ≥ 50%	POSITIVE

Note: if the sample control well (OD odd well) is greater than the mean value of the net positive control OD (net ODPC),the result cannot be interpreted.

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0.5 M) may be harmful if swallowed. It may cause sensitisation by skin contact (R22-43). Avoid contact with skin (S24-37).
4. Do not expose the substrate solution to bright light nor to oxidizing agents.
5. Decontaminate all reagents before elimination.