General Information

This diagnostic kit is designed to detect specific antibodies against the nucleoprotein (NP) of the Crimean Congo haemorrhagic fever virus (CCHFV) in bovine, ovine or caprine serum or plasma or other susceptible species.

Please contact IDvet for use in other species.

Description and Principle

Microwells are coated with recombinant purified CCHF nucleoprotein antigen (NP).

Samples to be tested and the controls are added to the microwells. Anti-NP antibodies, if present, form an antibody-antigen complex.

Plates are washed and a recombinant purified CCHF nucleoprotein antigen-HRP conjugate is added to the microwells. It fixes to the free Fab of the bound serum anti-NP antibodies.

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

The microplate is read at 450nm.

Note: This kit does not contain infectious material.

Kit Components

Reagents*
Microplates coated with CCHFV recombinant nucleoprotein
Concentrated Conjugate (10X), freeze-dried
Reconstitution Buffer
Positive Control
Negative Control
Dilution Buffer 14
Wash Concentrate (20X)
Substrate Solution
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. Wash, substrate and stop solutions can be used for the entire IDvet product range. Dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 10 µl, 100 µl, and 500 µl.
2. Disposable tips.
3. 96-well pre-dilution microplate
4. Distilled or deionized water.
5. Manual or automatic wash system.
6. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitization 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, for more detailed information.

Samples Preparation

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

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature 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/deionised 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 at info@id-vet.com

Reagent preparation and storage

Concentrated Conjugate (10X), freeze-dried:

Reconstitute the **freeze-dried Concentrated Conjugate 10X** with the **Reconstitution Buffer** supplied in the kit. **The volume to be added is mentioned on the label of each vial.** Wait approximately 5 minutes, and mix gently but thoroughly. Ensure complete solubilisation.

To guarantee constant performance once reconstituted, the **Concentrated Conjugate 10X** can be stored **up to 6 months at 5°C (± 3°C)**. For long-term storage, divide into **small aliquots** and store **at -20°C until the kit expiry date.** Each aliquot may undergo **no more than 3 freeze-thaw cycles.**

Testing Procedure

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

1. Add:
   - 50 µl of **Dilution Buffer 14** to each well.
   - 30 µl of the **Negative Control** to wells A1 and B1.
   - 30 µl of the **Positive Control** to wells C1 and D1.
   - 30 µl of each sample to be tested to the remaining wells.
2. Cover the plate and incubate **45 min** ± **4 min** at 21°C (± 5°C).
3. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 14.**
4. Empty the wells. Wash each well **5** times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
5. Add 50 µl of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well **5** 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 min** ± **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 N°8, to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean value of the Positive control O.D. (OD_{PC}) is greater than 0.350

$$OD_{PC} > 0.350$$

✓ the ratio of the mean values of the Positive and Negative controls (OD_{PC} and OD_{NC}) is greater than 3.

$$OD_{PC}/OD_{NC} > 3$$

Interpretation

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

$$S/P\,\% = \frac{OD_{sample}}{OD_{PC}} \times 100$$

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

- lesser than or equal to 30% are considered negative.
- greater than 30 % are considered positive.

Result	Status
S/P % ≤ 30%	NEGATIVE
S/P % > 30%	POSITIVE

Note: The IDSoft™ data analysis program is available free-of-charge. Please contact **support.software@id-vet.com** for more information.

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).

IDvet Innovative Diagnostics

Certified management system

ID Screen®
CCHF Double Antigen Multi-species

Double antigen ELISA for detection of antibodies against Crimean-Congo haemorrhagic fever virus (CCHFV) in serum or plasma from cattle, sheep, goats or other susceptible species.

For *in vitro* use

CCHFDA ver 0917 EN

008702

IDvet, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.id-vet.com - E-mail: info@id-vet.com