General Information

This diagnostic kit is designed to detect antibodies directed against the Bluetongue virus VP7 protein.

It can be used with sheep, goat, cattle, buffalo or deer serum or plasma.

Description and Principle

The wells are coated with the VP7 recombinant protein.

The samples to be tested and the controls are added to the microwells. The anti-VP7 antibodies, if present, form an antibody-antigen complex which masks the VP7 epitopes.

An anti-VP7-peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free VP7 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 solution appears which becomes yellow after addition of the stop solution.
- in the presence of antibodies, no coloration appears.

The microplate is read at 450nm.

Kit Components

Reagents*
Microplates coated with VP7 recombinant protein
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 2
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 10µl, 100µl and 500 µl.
2. Disposable tips.
3. Distilled or deionized water.
4. Manual or automatic wash system.
5. 96-well microplate reader.

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. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

Sample 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.

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.

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.

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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. Add:
   - 50 µl of **Dilution Buffer 2** 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 **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 2.**

   DO NOT EMPTY OR WASH THE PLATE
4. Add 100 µl of the **Conjugate 1X** to each well.
5. Cover the plate and incubate **30 min** ± **3 min** at 21°C (± 5°C).
6. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
7. Add 100 µl of the **Substrate Solution** to each well.
8. Cover the plate and incubate **15 min** ± **2 min** at 21°C (± 5°C) in the dark.
9. Add 100 µl of the **Stop Solution** to each well in the same order as in step No. 7, to stop the reaction
10. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

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

$$OD_{NC} > 0.700$$

✓ the ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is less than 0.3.

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

Interpretation

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

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

Samples presenting an S/N%:

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

Result	Status
S/N % < 40%	POSITIVE
S/N % ≥ 40 %	NEGATIVE

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®
Bluetongue Competition

Competitive ELISA for the detection of antibodies against the BTV VP7 protein in sheep, goat, cattle, buffalo or deer serum or plasma samples.

For *in vitro* use

BTC ver 1217 EN

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

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