General Information

This diagnostic kit is designed to detect anti-gP51 antibodies in bovine or buffalo sera (individual and pools of 10 samples)

Description and Principle

Wells are coated with gP51 antigen.

Samples to be tested and controls are added to the microwells. Anti-gP51 antibodies, if present, form an antibody-antigen complex masking the gP51 epitopes.

The conjugate anti-gP51-Peroxidase (HRP) is added to the microwells. It fixes to the remaining free gP51 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.

Kits Components

Reagents*
Microplates coated with BLV
Concentrated Coniugate (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. Components bearing the same name (*wash solution, dilution buffers*) can be used for the entire IDvet product range.

Materials required but not provided

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.

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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.

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.

Testing Procedure

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

SHORT INCUBATION (individual and pooled sera)

1) Add:
   - 80 µl **Dilution Buffer 2** to each well.
   - 20 µl of the Positive Control to wells A1 and B1.
   - 20 µl of the Negative Control to wells C1 and D1.
   - 20 µl of each sample to be tested to the remaining wells.
2) Incubate **45 min ± 4 min** at 21°C (± 5°C).

NIGHT INCUBATION (individual and pooled sera)

1) Add:
   - 190 µl **Dilution Buffer 2** to each well.
   - 10 µl of the **Positive Control** to wells A1 and B1.
   - 10 µl of the **Negative Control** to wells C1 and D1.
   - 10 µl of each sample to be tested to the remaining wells.
2) Incubate for **16** to **20** hours at 4°C (± 2°C).

The next steps are the same for the short and overnight incubations

3) Prepare the **Conjugate 1X** by diluting the **Concentrated conjugate** (**10X)** to 1/10 in **Dilution Buffer 2.**
4) Wash each well 3 **times** with approximately 300 µl of the **Wash Solution**. Avoid drying of the wells between washings.
5) Add 100µl of **Conjugate 1X** in each well.
6) Incubate for **30 min ± 3 min** at 21°C (± 5°C)
7) Wash each well **3* times** with approximately 300 µl of the **Wash Solution**. Avoid drying of the wells between washings.
8) Add 100 µl of the **Substrate Solution** to each well.
9) 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 order to stop the reaction.
11) Read and record the OD at 450 nm.

* Results can be influenced by the quality of washing. In order to improve results, we recommend 5 washes instead of 3 after the conjugate incubation.

Validation

The test is validated if:

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

$$OD_{NC} > 0.7$$

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

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

Interpretation

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

$$S/N\ \% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

Samples presenting a S/N% (short and overnight incubation, individual and pooled sera):

Result	Status
S/N % ≤ 50%	POSITIVE
50 < S/N % < 60%	DOUBTFUL
S/N % ≥ 60%	NEGATIVE

IDvet Innovative Diagnostics

Certified management system

ID Screen®
BLV Competition

Competitive Elisa for the detection of anti-gP51 antibodies in individual serum samples or pools of ten sera

Short or Night Incubation

For *in vitro* use

BLVC ver 0514 GB

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