General Information

This diagnostic kit is designed to detect antibodies directed against the Swine Vesicular Disease Virus (SVDV) in pig samples (serum or plasma). The method used by this kit is described in the OIE Manual for Terrestrial Animals, Chapter 2.8.9. It is based on the ELISA developed by Brocchi *et al.* (1) and is standardized to detect the Reference Serum produced by the OIE Reference Laboratory (Istituto Zooprofilattico Sperimentale, Brescia, Italy), calibrated on the Primary Reference Serum EU-RS4.

(1) Brocchi E., Berlinzani A., Gamba D. & De Simone F. (1995). Development of two novel monoclonal antibody-based ELISAs for the detection of antibodies and the identification of swine isotypes against swine vesicular disease virus. J. Virol. Methods, 52, 155-67.

Description and Principle

Wells are coated with SVDV antigen.

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

The conjugate Mab 5B7-peroxidase (HRP) is added to the microwells. It fixes to the remaining free SVDV 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 450nm.

Kit Components

Reagents*
Microplates coated with SVDV
Mab 5B7-Peroxidase Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 3
Dilution Buffer 1
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

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

1. The dilution buffer 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. 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 10 µl, 100 µl, and 500 µl.
2. Disposable tips.
3. 96 well 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 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 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 (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 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:
   - 60 µl of **Dilution Buffer 1** 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. Cover the plate and incubate **60 min** ± **6 min** at 37°C (± 3°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 **Conjugate 10X** to 1:10 in **Dilution Buffer 3.**
5. Add 100 µl of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 min** ± **3 min** at 37°C (± 3°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 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 #8, to stop the reaction.
11. Read and record the OD at 450 nm.

Validation

The test is validated if:

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

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

✓ the mean 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%):

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

Samples presenting a S/N%:

- less or equal to 45% are considered positive.
- greater than 45% and less or equal to 50% are considered doubtful
- greater than 50% are considered negative.

Result	Status
S/N% ≤ 45%	POSITIVE
45% < S/N% ≤ 50%	DOUBTFUL
S/N% > 50%	NEGATIVE

Note: The IDSoft™ data analysis program is available free-of-charge. Please contact, for more information, 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®
Swine Vesicular Disease Competition

Competitive ELISA for the detection of antibodies against the SVDV VP2 protein in serum or plasma

For *in vitro* use

0013205

SVDC ver 1014 EN

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