

PrioCHECK™ Porcine SVDV Ab Kit

ELISA for *in vitro* detection of antibodies against Swine Vesicular Disease Virus in plasma and serum of pigs

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.



WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at thermofisher.com. Wear appropriate protective eyewear, clothing, and gloves.

Introduction

Swine Vesicular Disease (SVD) is a highly contagious viral disease of pigs. Swine Vesicular Disease Virus (SVDV) is a member of the enterovirus genus in the family Picornaviridae. Although the disease is often mild in nature, it is an O.I.E. list A disease since it is clinically indistinguishable from Foot and Mouth Disease. For this reason outbreaks of SVD must be assumed to be Foot and Mouth Disease until laboratory diagnosis proves otherwise. The Applied Biosystems™ PrioCHECK™ Porcine SVDV Ab Kit meets the requirements for use in SVDV-screening programs. Based on a field study in the Netherlands a specificity of at least 99% and a sensitivity of 95% were found. In addition, the PrioCHECK™ Porcine SVDV Ab Kit is a convenient and simple test, which can be performed within 3 hours [1]. It is particularly suitable for use with automated ELISA systems, e.g. robots. All positive tested samples should be confirmed in the virus neutralization test.

Test principle

The PrioCHECK™ Porcine SVDV Ab Kit is a competitive double antibody sandwich test. Test Plates are coated with the mAb SVDV UK-72 followed by incubation with SVDV-antigen. Consequently, Test Plates of the kit contain SVDV-antigen captured by the coated mAb. The test is performed by dispensing the test sample and subsequently Conjugate (mAb SVDV UK-72/HRPO) is added to the wells of a Test Plate. After incubation, the plate is washed and the Chromogen (TMB) Substrate is dispensed. After incubation at room temperature (22±3°C) the color development is stopped. Color development measured optically at a wavelength of 450 nm shows the presence of antibodies directed against Swine Vesicular Disease Virus. The PrioCHECK™ Porcine SVDV Ab Kit is a single dilution test. Serum and/or plasma samples are tested in a 1:5 dilution.

Kit components

5 plate kit for 440 samples. Store kit at 5±3°C until expiry date. See kit label for expiry date. The shelf life of diluted, opened or reconstituted components is noted below, where appropriate.

Component	Description
1: Test Plate	Five Test Plates.
2: Conjugate (30x)	30x concentrated, dilute before use. One vial contains 1.5 mL Conjugate. Diluted conjugate is not stable, prepare just before use.
3: Conjugate Additive (30x)	Lyophilized. 30x concentrated, reconstitute before use. One vial contains 1.2 mL lyophilized Conjugate Additive. Shelf life of reconstituted Conjugate Additive: until expiry date stored at -20°C.
4: Washing Fluid (200x)	200x concentrated, dilute before use. One vial contains 60 mL Washing Fluid. Shelf life of washing solution: 1 week at 22±3°C.
5: Dilution Buffer	Ready-to-use. One vial contains 60 mL Dilution Buffer.
6: Demineralized Water	Two vials, each contains 10 mL Demineralized Water.
7: Blocking Reagent	Lyophilized. Five vials, each contains 2.0 mL lyophilized Blocking Reagent. Shelf life of reconstituted Blocking Reagent: until expiry date stored at -20°C.
8: Reference Serum 1	Ready-to-use. One vial contains 0.5 mL Reference Serum 1 (strong positive control).
9: Reference Serum 2	Ready-to-use. One vial contains 0.5 mL Reference Serum 2 (weak positive control).
10: Reference Serum 3	Ready-to-use. One vial contains 0.5 mL Reference Serum 3 (negative control).
11: Chromogen (TMB) Substrate	Ready-to-use. One vial contains 60 mL Chromogen (TMB) Substrate.
12: Stop Solution	Ready-to-use. One vial contains 60 mL Stop Solution.
Additional kit contents	<ul style="list-style-type: none"> Package Insert 5 plate sealers

Additional material required

Unless otherwise indicated, all materials are available through thermofisher.com.

Use	Description
General	Laboratory equipment according to national safety regulations.
Analysis of Results	Plate Reader. The reader has to have an appropriate filter set to read the plates at 450 nm.
Optional	Plate washer.

Test procedure

Precautions

- National Safety Regulations must be strictly followed.
- The PrioCHECK™ Porcine SVDV Ab Kit must be performed in laboratories suited for this purpose.
- Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Notes

To achieve optimal results with the PrioCHECK™ Porcine SVDV Ab Kit, the following aspects must be considered:

- The Test Procedure protocol must be strictly followed.**
- All reagents of the kit must be equilibrated to room temperature (22±3°C) before use.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Demineralized or water of equal quality must be used for the test.

Solutions to be made in advance

Conjugate Additive

Reconstitute the lyophilized Conjugate Additive (Component 3) with 1.2 mL Demineralized Water (Component 6). For partial use of the test kit, the reconstituted conjugate additive should be aliquoted into 240 µL (for a maximum of 5 tests) and stored at -20°C until expiry date.

Blocking Reagent

Reconstitute the lyophilized Blocking Reagent (Component 7) with 2 mL Demineralized Water (Component 6). Can be stored at -20°C until expiry date.

Reconstitution of the lyophilized reagents:

- 1. Equilibrate the vials to 22±3°C.
- 2. With the vial in an upright position, tap the vial gently against the worktop to ensure that the content is on the bottom of the vial.
- 3. Open the vial.
- 4. Add the required amount of Demineralized Water.
- 5. Replace the stopper on the vial and gently rotate the vial so that any remaining dry material is dissolved.
- 6. Allow the lyophilized material to stand for 15 minutes at 22±3°C.
- 7. Occasionally gently invert the vial (formation of foam should be avoided).

Conjugate dilution

Add 200 µL reconstituted conjugate additive, 1.8 mL reconstituted blocking reagent and 200 µL Conjugate (30x) (Component 2) to 3.8 mL Dilution Buffer (Component 5) and mix carefully.

Note: The diluted conjugate must be prepared just before use.

Washing solution

The Washing Fluid (Component 4) must be diluted 1:200 in demineralized water. A total volume of 12 liters of washing solution can be prepared from one vial Washing Fluid. Commercially available ELISA washers can be used. Stability of washing solution: 1 week stored at 22±3°C.

Incubation of samples, reference sera and conjugate

- 1. Dispense 50 µL of Dilution Buffer (Component 5) to wells G1 and H1 of the Test Plate (Component 1).
- 2. Dispense 40 µL of Dilution Buffer to all remaining wells of the Test Plate.
- 3. Dispense 10 µL of Reference Serum 1 (Component 8) to wells A1 and B1 of the Test Plate (= OD₄₅₀ blank).
- 4. Dispense 10 µL of Reference Serum 2 (Component 9) to wells C1 and D1 of the Test Plate.
- 5. Dispense 10 µL of Reference Serum 3 (Component 10) to wells E1 and F1 of the Test Plate.
- 6. Dispense 10 µL of the test samples in each of the remaining wells.
- 7. Add 50 µL of the diluted conjugate to all wells of the Test Plate.
- 8. Mix the contents of the plate manually or with a plate shaker.
- 9. Cover the plate with a plate sealer.
- 10. Incubate for 120±5 minutes at 22±3°C.

Incubation with Chromogen (TMB) Substrate

- 1. Empty the Test Plate and wash 6 times with 200 to 300 µL washing solution per well. Tap the plate firmly after the last washing (soaking the wells with washing solution is not needed).
- 2. Dispense 100 µL of the Chromogen (TMB) Substrate (Component 11) to all wells.
- 3. Incubate for 20 minutes at 22±3°C.
- 4. Add 100 µL of the Stop Solution (Component 12) to each well of the plate.
- 5. Mix the contents of the wells prior to measuring.

Reading of the test and calculating the results

- 1. Measure the optical density (OD) of the wells at 450 nm within 15 minutes after color development has been stopped.
- 2. Calculate the mean OD₄₅₀ value of the wells G1 and H1 (= OD₄₅₀ max).
- 3. Calculate the mean OD₄₅₀ value of wells A1 and B1 (Reference Serum 1 = OD₄₅₀ blank).
- 4. Calculate the corrected OD₄₅₀ value of the Reference Sera and all samples by subtracting the OD₄₅₀ blank.
- 5. Calculate the percentage inhibition (PI) of the Reference Sera 2, 3 and of the test samples according to the following formula:

PI = 100 - (corrected OD₄₅₀ test sample / corrected OD₄₅₀ max) × 100

Result interpretation

Validation criteria

- 1. The OD₄₅₀ max value must be >1.000.
- 2. The mean OD₄₅₀ of Reference Serum 1 (= OD₄₅₀ blank) must be <0.300.
- 3. Reference Serum 2 should have a percentage inhibition of >50.
- 4. Reference Serum 3 should have a percentage inhibition of <50.

The test samples of the concerning plate have to be tested again if any of the above mentioned criteria are not met.

Note: If the OD₄₅₀ max is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case warm the solution to 22±3°C or incubate up to 30 minutes.

Interpretation of the percent inhibition

PI = <50%	Negative	SVDV-specific antibodies are absent in the test sample.
PI = ≥50%	Positive	SVDV-specific antibodies are present in the test sample.

We do recommend retesting positive sera in the PrioCHECK™ Porcine SVDV Ab Kit. Subsequently, positive sera should be confirmed in the virus neutralization test.

Appendix - References

Chénard, G., Bloemraad, M., Kramps, J.A., Terpstra, C., Dekker, A (1998). Validation of a monoclonal antibody-based ELISA to detect antibodies directed against swine vesicular disease virus. *Journal of Virological Methods*, 75:105–112.

Customer and technical support

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- Worldwide contact telephone numbers
 - Order and web support
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)
- NOTE:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Revision history of Pub. No. MAN0013854 (English)

Rev.	Date	Description
A.0	15 October 2019	<ul style="list-style-type: none">• New document. Converted the legacy document (PrioCHECK SVDV Ab 7610205 v1.1_e.doc) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.• The product name was changed from PrioCHECK® SVDV Ab to PrioCHECK™ Porcine SVDV Ab Kit.

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