PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit

ELISA for in vitro detection of antibodies directed against the glycoprotein E (gE) of Pseudorabies Virus (Aujeszky's disease) in 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

Pseudorabies Virus (PRV), also called Aujeszky's disease virus, is an alpha herpes virus that causes a widespread infection of swine with an important economic impact on the swine industry. It can cause a wide range of clinical symptoms, including disorders of the nervous system, respiratory disease, reproductive failure, and death. The Applied Biosystems™ PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit detects antibodies in swine serum directed against the glycoprotein E (gE) of PRV and ignores antibody titers in pigs vaccinated with gE-deleted PRV marker vaccines. The presence of antibodies to gE indicates exposure to vaccines containing gE antigen and/or to field virus of different types.

The PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit enables to differentiate between infected pigs and vaccinated pigs, provided the pigs are vaccinated with viruses that fail to express gE. The PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit makes it possible to perform sero-epizootiological studies in vaccinated swine populations to detect pigs and pig herds infected with PRV. Consequently, the use of the PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit in conjunction with "gE-negative" vaccines can provide the basis for combined vaccination-eradication programs for Pseudorabies (Aujeszky's disease). Apart from this application to distinguish infected from vaccinated pigs, the PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit may also be employed to detect infected animals in a non-vaccinated pig herd.

Test principle

The PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit is a blocking ELISA. The reaction between an epitope, located on the glycoprotein E (gE) of PRV, and a specific monoclonal antibody (mAb) is blocked by specific antibodies that are present in the test sample. A Test Plate is coated with non-infectious PRV-antigen. ELISA buffer and undiluted swine serum are added to the wells (final serum dilution is 1:2). The plate is incubated for one hour at 37±1°C or overnight at 5±3°C. After washing of the plate, mAb-HRPO Conjugate is added and the plate is incubated again for one hour at 37±1°C. The plate is washed and Chromogen (TMB) Substrate is added. After 20 minutes, the color development is stopped by adding Stop Solution. The optical density (**OD**) is measured at 450 nm.

The kit also includes a ready-to-use Dynamic Validation Control (DVC). This low positive control allows the customer to:

- Start or maintain trend analysis of the PrioCHECK™ PRV gE 2.0 Antibody ELISA Kit.
- Dynamically elongate the time of substrate color development by using the software options and measurement performance at 650 nm.

Kit components

5 plate kit for 450 samples. Store kit at 5±3°C until expiry date. See kit label for actual expiry date. The shelf life of diluted, opened, or reconstituted components is noted below, when appropriate.

Component	Description
1: Test Plate	Five strip Test Plates packaged individually with a desiccant.
2: Conjugate (30x)	30x concentrated, dilute before use. One vial contains 2.5 mL Conjugate. Diluted conjugate is not stable, prepare just before use.
3: Dilution Buffer (2x)	2x concentrated, dilute before use. One vial contains 60 mL Dilution Buffer. ELISA buffer can be stored at 22±3°C up to 4 hours.
4: Washing Fluid (200x)	200x concentrated, dilute before use. One vial contains 60 mL Washing Fluid. Shelf life of the washing solution: 1 week at 22±3°C.
5: Negative Control	Ready-to-use. One vial contains 1.5 mL Negative Control (green).
6: Dynamic Validation Control	Ready-to-use. One vial contains 1.5 mL Dynamic Validation Control (orange).
7:Positive Control	Ready-to-use. One vial contains 1.5 mL Positive Control (red).
8: Chromogen (TMB) Substrate	Ready-to-use. One vial contains 60 mL Chromogen (TMB) Substrate.
9: Stop Solution	Ready-to-use. One vial contains 60 mL Stop Solution.
Additional kit contents	• Package Insert • 10 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.
Incubation	Microplate Incubator (reaching at least 50°C).
Analysis of results	Plate Reader e.g. Multiskan EX or equivalent. The reader has to have an appropriate filter set to read the plates at 450 nm.
Optional	Plate washer e.g. Tecan EIA Tray Washer or equivalent.

Test procedure

Precautions

- National Safety Regulations must be strictly followed.
- The PrioCHECK™ PRV gE 2.0 Antibody ELISA 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™ PRV gE 2.0 Antibody ELISA 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

ELISA buffer

The Dilution Buffer (Component 3) must be diluted 2 times in demineralized water and is sufficient to obtain a final volume of 120 mL of ELISA buffer.
The ELISA buffer can be stored at 22±3°C up to 4 hours.

Conjugate dilution

Prepare dilution of the Conjugate (30x) (Component 2) in ELISA buffer.
To perform a test with one plate, prepare 12 mL (add 0.4 mL Conjugate (30x) to 11.6 mL of ELISA buffer).
Note: The diluted conjugate must be prepared just before use.

Washing solution

The Washing Fluid (200x) (Component 4) must be diluted 1:200 in demineralized water and is sufficient to obtain a final volume of 12 liters washing solution.
Stability of washing solution: 1 week stored at 22±3°C.
Note: Commercially available ELISA washers can be used. If not available, washing of the plate can be done by dispensing 200 to 300 µL of washing solution to all wells of the plate. Subsequently, empty the plate and repeat as many times as prescribed. It is not necessary to soak the plate between washings. Tap the plate firmly after the last washing.

Incubation of test serum

1. Dispense 50 µL of ELISA buffer to all wells.
2. Dispense 50 µL of the Positive Control (Component 7) to wells A1 and B1 of the Test Plate (Component 1).
3. Dispense 50 µL of the Negative Control (Component 5) to wells C1 and D1.
4. Optional is to dispense 50 µL of the Dynamic Validation Control (Component 6) to wells E1 and F1.
5. Dispense 50 µL of test sample to one (single test) or two (duplicate test) adjacent wells of the Test Plate.
6. Seal the Test Plate with a plate sealer.
7. Shake the plate gently.
8. Incubate the plate for 60±10 minutes at 37±1°C preferably with 90% humidity or overnight (16–18 hours) at 5±3°C.

Incubation with the conjugate

1. Empty the plate after the sample incubation period and wash 6 times with 200 to 300 µL washing solution. Tap the plate firmly after the last wash cycle.
2. Dispense 100 µL of the diluted conjugate to all wells.
3. Seal the Test Plate with a plate sealer.
4. Incubate the plate for 60±20 minutes at 37±1°C preferably with 90% humidity.

Incubation with Chromogen (TMB) Substrate

1. Empty the plate after the conjugate incubation period and wash 6 times with 200 to 300 µL washing solution. Tap the plate firmly after the last wash cycle.
2. Dispense 100 µL of the Chromogen (TMB) Substrate (Component 8) to all wells.
3. Incubate the Test Plate 20 (15–30) minutes at 22±3°C.
   NOTE: The software option for dynamic elongation of the substrate color development can be used by measuring the optical density (**OD**) of the wells at 650 nm after minimal 10 minutes. Elongate the substrate incubation time when the results do not meet the validation criteria. Do not exceed a total of 30 minutes of substrate incubation.
4. Add 100 µL of the Stop Solution (Component 9) to all wells.
5. Mix the content of the wells of the Test Plate.

Note: Start the addition of Stop Solution 20 minutes after the first well was filled with Chromogen (TMB) Substrate. Add the Stop Solution in the same order and at the same pace as the Chromogen (TMB) Substrate was dispensed.

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 OD_{450} value of the Negative Control (wells C1 and D1). This is the OD_{450} **max** value.
3. Calculate the percent inhibition (**PI**) of the Positive Control, Dynamic Validation Control and of the **test samples** according to the formula below.

The OD_{450} of all samples is expressed as percent inhibition (**PI**) relative to the mean OD_{450} of the Negative Control (OD_{450} **max**) (wells C1 and D1).

$$PI = 100 - (OD_{450\ test\ sample} / OD_{450\ max}) \times 100$$

Result interpretation

Validation criteria based on measurement at OD_{450}

1. Mean OD_{450} of the Negative Control (wells C1 and D1) must be >0.90.
2. Percent inhibition (PI) of the Positive Control must be >65%.
3. Percent inhibition (PI) of the Dynamic Validation Control must be >45%.

Not meeting these criteria is reason to discard the results of that specific Test Plate.

Validation criteria based on measurement at OD_{650}

1. Mean OD_{650} of the Negative Control (wells C1 and D1) must be >0.40.
2. Mean OD_{650} of the Dynamic Validation Control (wells E1 and F1) must be >0.15.
3. Mean OD_{650} of the Dynamic Validation Control divided by the mean OD_{650} of the Negative Control must be <0.60 and >0.30.

Not meeting these criteria is reason to elongate the substrate incubation time of that specific Test Plate.

Note:

- If the mean OD_{450} of the Negative Control is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case preheat the solution to 22±3°C or incubate up to 30 minutes.
- If the mean OD_{450} of the Negative Control is above 2.000 a shorter incubation period with the Chromogen (TMB) Substrate is recommended.
- If the OD_{450} of a test sample is higher than the OD_{450} **max**, the percent inhibition can be interpreted as 0%.

Interpretation of the percent inhibition

PI = ≥35%	Positive	PRV gE specific antibodies are present in the test sample.
PI = <35%	Negative	PRV gE specific antibodies are absent in the test sample.

References

Van Oirschot J, Houwers D, Rziha H, Moonen P (1988). *J Virol Methods* 22 (2–3):191–206.

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**
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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	Date	Description
A.0	30 April 2019	New document. Converted the legacy document (MAN0013819_7589010_UG_en.pdf) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.