PrioCHECK® PPV Ab

ELISA for *in vitro* detection of antibodies directed against Porcine Parvo Virus in serum of pigs

2 plate kit for 176 samples

Version 1.1_e

Package Insert

For *in-vitro* veterinary diagnostic use only
Store at 5±3°C
Product No.: 7588981

Introduction

Porcine Parvovirus (PPV) is a small single-stranded DNA virus, belonging to the family of the parvoviridae. The virus is a ubiquitous infectious agent of pigs causing reproductive failures in pig herds, and is of economic concern to pig breeders worldwide. The virus is capable of crossing the placenta. The consequences of an infection with PPV are dependent on the immune status of the dam on the stage of gestation at which infection occurs. In the non-immune dam, PPV infections, which occur prior to 35 days gestation, can lead to embryonic death. After approximately 70 days, when fetuses are immunocompetent, the porcine fetus is capable of mounting an immune response by producing antibodies to PPV and may survive infection. This usually results in birth of healthy, serologically positive piglets.
The PrioCHECK® PPV Ab detects specific antibodies as early as seven days after primary infection. The antibody titer reaches a plateau at approximately 10 days after infection.
By titration of paired serum samples, collected from a herd at an interval of several weeks, the infection status can be monitored. Based on this information, decisions can be made on control of the infection by vaccination.

Test Principle

The PrioCHECK® PPV Ab ELISA is a single dilution test. Serum samples are tested in a 1:5 dilution. Dilutions of test serum are pre-incubated with PPV antigen. During this incubation, specific antibodies, if present, will form an immune complex with the antigen. After this pre-incubation, the mixture is transferred to an antibody-coated test plate. During a subsequent incubation in the test plate, free PPV antigen in the mixture is bound to the antibody-coated well, whereas antigen which previously has formed an immune complex with antibodies in the test serum (= blocked antigen) is not bound. After washing away unbound reagents, conjugate is added and this will react with the bound antigen. After incubation, wells are washed again and Chromogen (TMB) Substrate is added.
Finally, color development is stopped and measured at a wavelength of 450 nm.

Kit Components

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. Chemical hazard data are available in section "Safety Regulations and R&S Statements" (Appendix II).

Component 1
Test Plate
Two strip Test Plates.

Component 2
Conjugate (30x)
(30x concentrated, dilute before use)
One vial contains 1.5 ml Conjugate.
Diluted conjugate is not stable, prepare just before use.

Component 3
Dilution Buffer (5x)
(5x concentrated, dilute before use)
One vial contains 60 ml Dilution Buffer.
Shelf life of dilution buffer working solution: 5 hours at 22±3°C.

Component 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.

Component 5
Demineralized Water
One vial contains 10 ml Demineralized Water.

Component 6
Horse Serum (lyophilized)
One vial contains 3.5 ml lyophilized Horse Serum.
Shelf life of reconstituted horse serum: until expiry date at -20°C.

Component 7
Antigen (lyophilized)
Two vials, each contains 6.0 ml lyophilized Antigen.
Shelf life of reconstituted antigen: in aliquots until expiry date at -20°C.

Component 8
Reference Serum 1 (Ready-to-use)
One vial contains 1.0 ml Reference Serum 1 (positive control).

Component 9
Reference Serum 2 (Ready-to-use)
One vial contains 1.0 ml Reference Serum 2 (negative control).

Component 10
Chromogen (TMB) Substrate (Ready-to-use)
One vial contains 60 ml Chromogen (TMB) Substrate.

Component 11
Stop Solution (Ready-to-use)
One vial contains 60 ml Stop Solution.

Additional Kit Contents:
- Package Insert
- 1 lid to cover the plate during incubation
- Certificate of analysis

Additional Material Required

General:
Laboratory equipment according to national safety regulations.

Dummy plates:
Dummy plates for diluting of test sera with the reconstituted antigen. We advice U-bottom shaped plates (Greiner, art. nr. 6501101). However, also other non binding plates or tubes can be used.

Incubation:
Microplate Incubator (reaching at least 50°C).

Analysis of Results:
Plate Reader e.g. Multiscan 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 guidelines for working with animal samples must be strictly followed. The PrioCHECK® PPV Ab 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.

Chemical hazard data are available in section "Safety Regulations and R&S Statements" (Appendix II).

Notes
To achieve optimal results with the PrioCHECK® PPV Ab, 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

Dilution buffer working solution
The concentrated Dilution Buffer (5x) (Component 3) must be diluted 1/5 in demineralized water.
Stability of dilution buffer working solution: 5 hours at 22±3°C.

Horse Serum
Reconstitute[1] the Horse Serum (Component 6) with 3.5 ml Demineralized Water (Component 5). After reconstitution, store at -20°C until expiry date.

Conjugate buffer
To perform a test with two strips add to 1.9 ml dilution buffer working solution, 0.1 ml reconstituted horse serum (final concentration of horse serum is 5% (v/v)). Unused conjugate buffer can be stored at 5±3°C for up to 24 hours.

Conjugate dilution
Dilute the Conjugate (30x) (Component 2) 1/30 with conjugate buffer. Prepare 1.8 ml to perform a test with two strips.
Note: The diluted conjugate must be prepared just before use.

Antigen
Reconstitute[1] the Antigen (Component 7) with 6 ml dilution buffer working solution. For partial use of the test kit the vial reconstituted antigen should be divided in 1.0 ml aliquots (for a maximum of 6 test runs per plate). Avoid multiple freezing and thawing and store the aliquots in small vials at -20°C.

[1] Reconstitution of lyophilized reagents should be performed as follows:
- Equilibrate the vials to 22±3°C.
- 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.
- Open the vial.
- Add the required amount of demineralized water.
- Replace the stopper on the vial and gently rotate the vial so that any remaining dry material will be dissolved.
- Allow the lyophilized material to stand for 15 minutes at 22±3°C.
- Occasionally gently invert the vial (formation of foam should be avoided).

Washing solution
The Washing Fluid (200x) (Component 4) must be diluted 1/200 in demineralized water and is sufficient for a final volume of 12 liters of washing solution.
Stability of washing solution: 1 week stored at 22±3℃.

Note: Commercially available ELISA washers can be used. If not available, washing of the plates can be done by hand by dispensing 200 - 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. Tap the plate firmly after the last washing.

PRE-INCUBATION OF TEST SAMPLES AND ANTIGEN

1.1 Mix 96 µl dilution buffer working solution with 24 µl Reference Serum 1 (Component 8) and 120 µl reconstituted antigen.
1.2 Mix 96 µl dilution buffer working solution with 24 µl Reference Serum 2 (Component 9) and 120 µl reconstituted antigen.
1.3 Mix 12 µl of each serum sample with 48 µl dilution buffer working solution and 60 µl reconstituted antigen.
1.4 Incubate for 60±5 minutes at 37±1℃.

INCUBATION IN TEST PLATE

2.1 Label each strip of the Test Plate (Component 1) with a marker pen.
2.2 Dispense 100 µl of dilution buffer working solution to wells A1 and B1.
2.3 Dispense 50 µl of dilution buffer working solution to wells C1 and D1.
2.4 Add 50 µl of reconstituted antigen to wells C1 and D1 and mix.
2.5 Dispense 100 µl of reference serum 1 - antigen mixture to wells E1 and F1.
2.6 Dispense 100 µl of reference serum 2 - antigen mixture to wells G1 and H1.
2.7 Dispense 100 µl of the test sample - antigen mixture to the remaining wells.
2.8 Seal the Test Plate and incubate for 120±5 minutes at 37±1℃.

INCUBATION WITH CONJUGATE

3.1 Empty the Test Plate and wash the plate 6 times with 200 to 300 µl washing solution. Tap the plate firmly after the last wash cycle.
3.2 Dispense 100 µl of diluted conjugate to all wells.
3.3 Seal the Test Plate and incubate for 60±5 minutes at 37±1℃.

INCUBATION WITH CHROMOGEN (TMB) SUBSTRATE

4.1 Empty the Test Plate after the incubation period and wash the plate 6 times with 200 to 300 µl washing solution. Tap the plate firmly after the last wash cycle.
4.2 Dispense 100 µl of Chromogen (TMB) Substrate (Component 10) to all wells.
4.3 Incubate the plate for 15 minutes at 22±3℃.
4.4 Add 100 µl of Stop Solution (Component 11) to all wells.
4.5 Mix the content of the wells prior to measuring.

Note: Start the addition of Stop Solution 15 minutes after the first well was filled with Chromogen (TMB) Substrate. Add the Stop Solution in the same order and the same pace as the Chromogen (TMB) Substrate was dispensed.

READING OF THE TEST AND CALCULATING THE RESULTS

5.1 Measure the optical density (OD) of the wells at 450 nm within 15 minutes after color development has been stopped.
5.2 Calculate the mean OD_{450} value of wells A1 and B1 (=OD_{450} blank).
5.3 Calculate the corrected OD_{450} value of reference sera 1, 2 and of the serum samples by subtracting the mean OD_{450} blank.
5.4 Calculate the mean of the corrected OD_{450} of wells C1 and D1 (=OD_{450} max).
5.5 The percentage inhibition (PI) is calculated according to the following formula:

Note: The corrected OD_{450} of all samples is expressed as percent inhibition (PI) relative to the corrected mean OD_{450} max.

$$PI = 100 - \left[\frac{\text{corrected } OD_{450} \text{ test sample}}{\text{corrected } OD_{450} \text{ max}} \right] \times 100$$

RESULT INTERPRETATION

Validation criteria
6.1 The mean OD_{450} blank (wells A1 and B1) should be <0.300.
6.2 The corrected OD_{450} max (wells C1 and D1) must be >1.000.
6.3 The PI of Reference Serum 1 (positive control) must be >50%.
6.4 The PI of Reference Serum 2 (negative control) must be <50%.
6.5 Not meeting any of these criteria is reason to discard the results of that specific test plate.

Note: If the corrected OD_{450} max is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case warm the solution to 22±3℃ or incubate up to 30 minutes.
If the corrected mean OD_{450} max is above 2.000 a shorter incubation period with the Chromogen (TMB) Substrate is recommended.

Interpretation of the percentage inhibition

PI = ≥50%
Test sample is positive for PPV specific antibodies.

PI = <50%
Test sample is negative for PPV specific antibodies.

Appendix I

Notice
This manual is believed to be complete and accurate at the time of publication. In no event shall Prionics AG be liable for incidental or consequential damage in connection with or arising from the use of this manual.

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Prionics AG warrants its products will meet their applicable published specification when used in accordance with their applicable instructions and within the declared products life time. Prionics AG makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications and descriptions of Prionics AG products appearing in Prionics AG published catalogues and product literature may not be altered except by express written agreement signed by an officer of Prionics AG. Representation, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.
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Prionics AG and Prionics Lelystad B.V. are ISO 9001:2000 certified companies.

Appendix II

Safety Regulations and R&S Statements
National Safety Regulations must be strictly followed.

Component 1
Test Plate
Hazard Code: This product is not classified according to EU regulations.

Component 2
Conjugate (30x)
Hazard Code: This product is not classified according to EU regulations.

Component 3
Dilution Buffer (5x)
Hazard Code: This product is not classified according to EU regulations.

Component 4
Washing Fluid (200x)
Hazard Code: This product is not classified according to EU regulations.

Component 5
Demineralized Water
Hazard Code: This product is not classified according to EU regulations.

Component 6
Horse Serum (lyophilized)
Hazard Code: This product is not classified according to EU regulations.

Component 7
Antigen (lyophilized)
Hazard Code: This product is not classified according to EU regulations.

Component 8
Reference Serum 1 (Ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 9
Reference Serum 2 (Ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 10
Chromogen (TMB) Substrate (Ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 11
Stop Solution (Ready-to-use)
Hazard Code: R35: Causes severe burns.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label on vial).

Appendix III

References
(1) F. Westenbrink, M.A. Veldhuis and J.M.A. Brinkhof Journal of Virological Methods, 23 (1989) 169-178.

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