For Research Use Only Not for Diagnostic Use

PPV ELISA Assay

Catalog# PK002C

Introduction

Porcine Parvovirus (PPV) is a small single stranded DNA virus that is endemic worldwide. Structurally, PPV is non-enveloped and spherical in shape. PPV infection is a major source of reproductive failure in pig populations, which includes ailments such as embryonic/fetal death, still births, and weak piglets.

Principle of the Assay

Microtitration wells coated with recombinant PPV VP2 capsid antigen are exposed to test specimens, which may contain antibodies to PPV. After an incubation period, unbound components in the test sample are washed away. Specifically bound PPV antibody reacts with an anti-swine IgG peroxidase conjugate during a second incubation period. Following a second wash cycle, the specifically bound peroxidase conjugate is detected by reaction with the Substrate Solution, 2,2' Azino-di[3-ethyl-benzthiazoline-sulfonate] ABTS. The colorimetric assay is measured spectrophotometrically (405 nm) to indicate the level of PPV positive antibody present in a sample.

Kit Presentation

Materials Supplied
The reagents supplied in this pack are for Research use only.

1	Antigen coated microwell strips 2 strip holders containing: 6 positive viral antigen coated strips 6 negative antigen coated strips Alternating (+) and (–) antigen strips	2 plates 96 (+) wells 96 (-) wells
2	Positive Swine PPV Control Serum	1 vial of 1mL
3	Negative Swine PPV Control Serum	1 vial of 1mL
4	Anti-Swine IgG Peroxidase Conjugate	2 x 12 mL
5	Wash Buffer (20x concentrated). Tris buffered saline pH 7.8-8.0, containing 0.05% Tween 20. Must be diluted before use.	2 x 60 mL
6	ABTS Substrate Solution	2 x 12 mL
7	Stop Solution.	1 x 10 mL
8	Sample Diluent	2 x 30mL

Additional Requirements for Manual Processing
> Disposable tip micropipettes to deliver volumes of 5 µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
> Distilled or deionized water.
> 37 (±1)º C incubator.
> Clean, disposable plastic/ glass test tubes, approximate capacities 5 mL and 10 mL.
> Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder,1 mL, 5 mL, and 10 mL glass pipettes.
> Absorbent paper towels.
> Automatic microtitration plate washer or laboratory wash bottle.
> Microtitration plate reader with 450 nm filter.
> Latex gloves, safety glasses and other appropriate protective garments.
> Biohazard infectious waste containers.
> Safety pipetting devices for 1 mL or larger pipettes.
> Timer.

Automatic, or Semi-automatic Processing

The Swine PPV Assay may be used with a variety of automatic or semi-automatic processors/liquid handling systems. It is essential that any such system is qualified, before it is used routinely, by demonstrating that the Swine PPV Assay results obtained using the automatic processor are equivalent to those obtained for the same specimens using the manual test method. Subsequently the automatic processor should be periodically re-qualified.

Storage and Stability

Upon receipt, the PPV Assay should be stored at 2-8°C, and should not be used beyond the expiration date on the label. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained. Unused strips should be returned to their original foil pouch along with the sachet of desiccant. Opened pouches should be securely resealed.

The working strength Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the working strength buffer becomes visibly cloudy or develops precipitate during the 3 weeks, do not use it.

Indications of Deterioration
The Swine PPV Assay may be considered to have deteriorated if:
1. The kit fails to meet the required criteria for a valid test (see interpretation of results).
2. Reagents becoming visibly cloudy or develop precipitate. *Note*: Concentrated Wash buffer, when cold, normally develops crystalline precipitates, which re-dissolve on heating at 37°C.
3. The Substrate Solution turns dark green. This is likely to be caused by chemical contamination of the Substrate Solution.

Warnings and Precaution

Safety
1. The reagents supplied in this kit are for ***Research use only***.
2. Caution: All blood products should be treated as potentially infectious. Essential precautions can be summarized as follows**:**
   >do not pipette by mouth.
   >Wear disposable gloves during all specimen and assay manipulations.
   >Avoid use of sharp or pointed liquid handling devices, which may puncture skin.
   >Do not smoke, eat or drink in the laboratory work area.
   >Avoid splashing of liquid specimens and reagents and the formation of aerosols.
   >Wash hands thoroughly on completion of a manipulation.
3. The Swine PPV kit contains reagent systems, which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.
4. The Substrate Solution and Stop Solution in this kit contain ingredients that can irritate the skin and cause eye damage. Handle them with care and wear suitable protective clothing and eye/face protection. In case of contact with skin or eyes, immediately flush the affected area with plenty of water. For eyes, obtain medical attention.

Procedural

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use Swine PPV Assay kits after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents. Always use fresh pipette tips when drawing from stock reagent bottles.
4. Always use clean, preferably disposable, glassware for all reagent preparation.
5. Allow foil bags to warm to room temperature before opening. This avoids condensation on the inner surface of the bag, which may contribute to a deterioration of coated strips intended for future use.
6. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. Follow manufacturer's recommendations for automatic processors.
7. Always keep the upper surface of the microtitration strips free from excess fluid droplets. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
8. Do not allow the wells to completely dry during an assay.
9. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.
10. Automatic or semi-automatic EIA processors or liquid handling systems should be qualified specifically for use with PPV Assay by demonstration of equivalence to the manual processing methods.
11. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers and thermometers are regularly calibrated according to the manufacturer's instructions.
12 Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

Method of Use

Specimen Collection, Preparation and Storage

A. The PPV Assay is intended for use with blood serum. Obtain blood and allow clot to form. Insoluble materials should be removed by centrifugation. Remove the serum aseptically. Serum samples should be refrigerated as soon as possible after collection. If not assayed within 48 hours, the samples should be aliquoted and frozen. Avoid repeated freezing/thawing of samples. Samples should not contain sodium azide.

B. Dilute the serum 1:50 in Sample Diluent. For example: add 5 ul of serum sample to 245 ul of 1X Sample Diluent. If not assayed immediately, diluted samples should be stored at -20°C or below.

Rinse Cycle

Efficient rinsing to remove un-complexed components is a fundamental requirement of enzyme immunoassay procedures. The PPV assay utilises two standard five-rinse cycles. Automatic plate washers may be used provided they meet the following criteria:

1. All wells are completely aspirated.
2. All wells are filled to the rim (350 µL) during the rinse cycle.
3. Wash buffer is dispensed at a good flow rate.
4. The microtitration plate washer must be well maintained to prevent contamination from previous use. Manufacturer's cleaning procedures must be followed diligently

For each rinse cycle the machine should be set to five consecutive washes. On completion of the cycle, invert the microtitration plate and tap firmly on absorbent paper towels. Check for any residual Wash buffer in the wells and blot dry the upper surface of the wells with a paper towel.

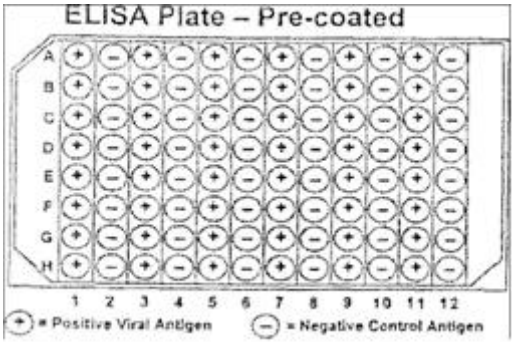
Alternatively, the following manual system may be employed:

1. Aspirate well contents using a vacuum line fitted with a trap.
2. Fill all wells to the brim with Wash buffer dispensed from a squeeze-type laboratory wash bottle.
3. Aspirate all wells.
4. Repeat steps 2 and 3, four additional times.
5. Invert the microtitration plate and tap firmly on absorbent paper towels.

Preparation for the Assay

1. Review the complete instructions before performing the test.
2. Strips of the ELISA plate are removable. Remove unused strips and store as described in "Storage and Stability Instructions." Before testing begins, the user should inspect the ELISA strip holders and ensure that all strips are secure. A white stabilizer residue is normally observed in the bottom of unused wells.
3. Strip holders should be handled with care to ensure that no strip is dislodged during testing. It is recommended that each strip be numbered with a laboratory marker prior to use. Additionally, since the strips are pre-coated with positive viral antigen and negative control antigen (alternating 6 positive and 6 negative antigen-coated strips/strip holder), it is recommended that each strip be labeled with a "+" or "-" to indicate type of antigen coat in each well.

NOTE: Assembled strip holders always start with a positive antigen (+) coated strip. Subsequent strips alternate between negative control (-) antigen and positive (+) antigen, so that strips 1,3,5,7,9 and 11 are pre-coated with positive (+) antigen and strips 2,4,6,8, 10 and 12 are pre-coated with negative (-) control antigen. A schematic representation of this is below.



Avoid touching bottom surfaces of wells, as this may affect readings. ELISA strips must be used only once. Strip holders may be used again.

4. Dispose of all used materials as biohazardous waste.
5. A new pipette tip must be used for each sample, never touching pipette tip to the bottom of the well. If plastic troughs are used, ensure that they have a dedicated purpose (do not use the same trough for Peroxidase Conjugate and ABTS Peroxidase Substrate).

Wash Buffer

Prepare working-strength Wash buffer by diluting 1-part concentrate with 19-parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs. Each row of 8 wells may be adequately washed with 150 mL of working strength buffer.

Assay Procedure

All samples and Controls should be tested on both the positive viral antigen and the negative control antigen wells. Use the enclosed record sheet to identify the location of each serum and type of strip (+ or - antigen) used in the test.

1. Make a 1:50 dilution of the test serum in 1X Sample Diluent in a small dilution tube and mix well. EXAMPLE: Add 5 ul of serum to 245 ul of 1X Sample Diluent.
2. Fit the strip holder with the required number of pre-coated Positive Viral Antigen and Negative Control Antigen strips. Mark the appropriate strips with a (+) or (-). Allow one well to be used for the Negative Control Sera and one well for the Positive Control Sera.
3. Pipette 100 ul each of the diluted serum sample, the Negative Control and Positive Control into the appropriate (+) and (-) marked wells.
4. Cover the wells and incubate at 37°C for 45± 1 minutes.
5. After incubation, wash each well five (5) times with 1X Wash Solution (refer to rinse cycle).
6. Pipette 100 ul of liquid, ready-to-use Peroxidase Conjugate into each test well. Cover the wells and incubate at 37°C for 45± 1 minutes.
7. After incubation, wash each well five (5) times with 1 X Wash Solution (refer to rinse cycle).
8. Pipette 100 ul of liquid, ready-to-use ABTS Substrate Solution into each test well.
9. Incubate the plate at room temperature (20 - 25°C) for 30 minutes. Do not cover the plate.
10. Blank the micro reader on air and read the absorbance of the colorimetric reaction in each well at 405nm.
11. If the plate is not read immediately, pipette 25 ul of Stop Solution into each test well. Read the plate at 405 nm within 15 minutes.

Interpretation of Results

1. It is recommended that each laboratory establish their own criteria for performance of these Research Reagents.
2. In our quality control testing, we use the following criteria:
    a. The Negative Control Serum, after subtracting the absorbance in the negative control antigen well, should produce a net absorbance on the Positive Viral Antigen of ≤ 0.250 at 405 nm.
    b. The Positive Control Serum, after subtracting the absorbance in the negative control antigen well, should produce a net absorbance on the Positive Viral Antigen of ≥ 0.600 at 405 nm.
    c. A sample may be considered positive by the following criteria: Determine the difference (Δ) between the sample absorbance at 405 nm on the Positive Viral Antigen well and the absorbance at 405 nm on the Negative Control Antigen well. **This difference (Δ) should be greater than or equal to 0.300 for a sample to be considered positive.**

Example # 1: Positive Sample
Given a sample absorbance of **1.101** at 405 nm on the Positive Viral Antigen well and a sample absorbance of **0.190** at 405 nm on the Negative Control Antigen well.
The difference (Δ) between the above absorbances is **0.911.**
This difference is greater than or equal to **0.300**. This sample is considered Positive.

Example # 2: Negative Sample
Given a sample absorbance of **0.347** at 405 nm on the Positive Viral Antigen well and a sample absorbance of **0.319** at 405 nm on the Negative Control Antigen well.
The difference (Δ) between the above absorbances is **0.028.**
This difference is less than 0.300. This sample is considered negative.

Limitations of Use

1. Assay values determined using assays from different manufacturers or different methods may not be used interchangeably.
2. The performance characteristics have not been established for any matrices other than swine serum

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