TECHNICAL MANUAL

Porcine Parvovirus Antibodies ELISA Kit

- **SKU CODE:** ADES0059
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Indirect
- **RUO:** Research-Use-Only

Revised: 11/2025

Porcine Parvovirus Antibodies ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key Features

Detection Method:

Indirect, Qualitative

Sample Type:

Serum and plasma

Reactivity:

Porcine

Range:

-

Sensitivity:

-

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit and/or components as described in section 4. Date of expiration is on the ELISA Box label.

3. Product Description

The Assay Genie Porcine Parvovirus Antibodies ELISA Kit includes an HRP-conjugated detection reagent, assay buffers, and an ELISA microtiter plate pre-coated with recombinant PPV antigen. The assay follows the enzyme-linked immunosorbent assay (ELISA) principle for the qualitative detection of PPV -specific antibodies in porcine serum.

During the assay procedure, controls and test samples are added to the antigen-coated wells, where PPV antibodies (if present) bind to the immobilized viral antigen. After incubation, unbound components are removed by washing. An HRP-conjugated secondary reagent is then added, which binds to the antigen-antibody complexes formed in the wells. Following a second wash to eliminate excess conjugate, a chromogenic substrate is added. The resulting enzymatic reaction produces a yellow color, with intensity proportional to the amount of PPV -specific antibody present in the sample.

The reaction is terminated by adding Stop Solution, and absorbance is measured at 450 nm with a 630 nm reference using a microplate reader. The resulting OD values are used to determine whether PPV antibodies are present in the tested samples.

4. Kit Contents

No	Component Name	Specifications	Storage
1	ELISA Microplate	96 wells	Store at 2-8℃. Avoid freeze.
2	Dilution plate	96 wells	
3	HRP Conjugate	12 mL	
5	Sample Diluent	50 mL	
6	10x Concentrated Wash Buffer	50 mL	
7	Substrate Reagent A	6 mL	
8	Substrate Reagent B	6 mL	
9	Stop Solution	6 mL	
10	Positive Control	0.8 mL	
11	Negative Control	0.8 mL	
12	Plate Sealer	3 pieces	-
13	Sealed Bag	1 piece	
14	Technical Manual	1 copy	

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. This kit is ideal for research purposes only and not for diagnostics or therapeutic uses.
2. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date.
3. Allow all reagents and samples to reach room temperature before use.
4. Ensure unopened and unused plate is kept dry to avoid contamination.
5. Before using the kit, centrifuge tubes to spin down standard and/or antibody.
6. Prepare all reagents, samples and standards as directed in this manual.
7. Duplicate wells are recommended for both standard and sample testing.
8. Do not let the microplate wells dry during assay.
9. Maintain consistent incubation times and temperatures as variations can affect results.
10. Do not reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.

6. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

General Considerations

To prevent denaturation or degradation of target proteins, it is recommended to process samples promptly and store them under appropriate conditions.

- **Storage Conditions**:
    - **Short-term**: 2-8 °C for up to 5 days.
    - **Medium-term**: -20 °C for up to 6 months.
    - **Long-term**: -80 °C or cryopreservation in liquid nitrogen.
- **Thawing Protocol**: Frozen samples should be thawed rapidly in a 15-25 °C water bath to minimize ice crystal-induced damage. Thawed samples can be analyzed immediately or stored temporarily at 2-8 °C.
- **Freeze-Thaw Cycles**: Repeated freeze-thaw cycles should be strictly avoided due to their detrimental effect on protein stability.

Blood-Derived Samples

- **Serum:** Allow whole blood to coagulate at room temperature (2 h) or 2-8 °C overnight. Centrifuge at 1000 × g for 20 min and collect the supernatant. Store or use immediately.
- **Plasma:** Collect in anticoagulant tubes (EDTA, citrate, or heparin), mix gently, and centrifuge within 30 min at 1000 × g, 2-8 °C for 15 min. Store or assay as needed.

- **Anticoagulant Guidance:** The document provides detailed recommendations on the selection and properties of EDTA, citrate, and heparin for various analytical requirements.

7. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350 µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350 µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute.

Note: *Set the height of the needles; be sure the fluid can be taken up completely.*

Sample Dilution Guidelines

Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer.

Note: *Dilution may be necessary to minimize matrix effects. However, if the target concentration in the sample is very low, the pre-treated sample can be added directly to the assay without dilution.*

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

A. Diluted serum:

- Dilute the sample with the Sample Diluent at 1:50 (4 µL sample and 200 µL of sample diluent, mix fully). The positive/negative control do not need to be diluted.

B. Wash Buffer:

- The 10×Concentrated Wash Buffer should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:9.

8. Assay Procedure

1. **Plate Setup:** Set test sample positive control and negative control wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. ***Note:*** *Dispense all solutions directly to the bottom of the plate wells, avoiding contact with the well walls. Take care to prevent foaming during the addition of solutions.*
2. **Samples & Control Positive/Negative Loading:** Aliquot 100 µl of µl diluted serum samples and controls into the designated wells. **Note:** *Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.*
3. **First Incubation**: Seal the plate with a cover and incubate at 37 °C for 30 mins. Protect from light.
4. **Wash:** Aspirate or decant the solution from the plate and add 250 µL of wash buffer and was. Repeat wash procedure for 5 times, 30 s intervals/time. Aspirate the solution from each well and clap the plate on absorbent filter paper to dry. ***Note:*** *A microplate washer can be used in this step and other wash step.*
5. **HRP Conjugate Solution Addition:** Add 100 µL of HRP Conjugate Solution to each well.
6. **Second incubation:** Cover with a plate seal and incubate for 30 min at 37°C.
7. **Wash**: Repeat the wash process five times as conducted in step 4.
8. **Substrate Reagent Addition and Colour Development**: Add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B into each well. Cover the plate sealer and mix thoroughly, incubate at 37°C for 10 min in shading light. (***Note****: This incubation time is for reference only, the optimal time should be determined by the end-user. DO NOT exceed 30 minutes).*
9. **Stop Reaction**: Add 50 µl of Stop solution into each well and mix thoroughly. The colour changes into yellow immediately.
10. **OD Measurement**: Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. Read the results within 10 min.

9. Results

Reference value

Normally, the average OD of negative control < 0.2 and the OD of positive control > 0.6.

Results Interpretation

Cutoff value = 0.15 + OD Negative control

1. Positive result: OD Sample > Cutoff value
2. Negative result: OD Sample < Cutoff value
3. If the value of the negative control well is less than 0.05, it shall be calculated as 0.05.
4. Unimmunized animal: positive result indicates that it may be infected with PPV. Immunized animal: The antibody levels at the time of the sample were monitored and recorded, and the distribution of antibody levels and the trend of immune status of the flock were analyzed based on the results.

Limitations

1. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.