Porcine Epidemic Diarrhea Virus IgA Ab ELISA

BIONOTE

BIONOTE PED IgA Ab ELISA

■ Principle of the Test

The BIONOTE PED IgA Ab ELISA contains a microplate, which is pre-coated with PEDV antigen on the well. For testing, the ELISA plate is incubated with diluted samples and controls for 60 minutes at 37 ℃. During the first incubation, anti-PEDV IgA Ab in the sample binds onto the PEDV antigen coated on the well. After the incubation and washing step, rabbit anti-pig IgA-HRP conjugate is dispensed into the wells and incubated for 30 minutes at 37 ℃. Following this incubation, all unbound materials are removed by wash step through aspiration. The enzyme-linked complex is then revealed by the addition of substrate. The enzyme activity found on the well will thus be directly proportional to the anti-PEDV IgA Ab in the sample. The reaction is stopped by the addition of the stop solution, and colorimetric reading will be performed using a spectrophotometer at 450 nm with a reference wavelength at 620 nm. The highly specifically selected PEDV antigens are used as capture material in the test. These enable the BIONOTE PED IgA Ab ELISA to identify anti-PEDV IgA Ab in pig colostrums, with a high degree of accuracy.

■ Materials Provided

1	Antigen Coated Microplate	96 wells/plate, configured in twelve 1x8 strip. PED antigens coated on the wells.
2	Negative Control	Colostrums and Proclin 300 (0.05%).
3	Positive Control	Purified IgA to PEDV antigen in colostrums and Proclin 300 (0.05%).
4	Sample Diluent	Phosphate buffered saline and Proclin 300 (0.05%).
5	20X Washing Solution	PBS-Tween 20 and Proclin 300 (0.05%).
6	101X Enzyme Conjugate	Rabbit anti-pig IgA-HRP, BSA, and Proclin 300 (0.05%).
7	Conjugate Diluent	Phosphate buffered saline, BSA, and Proclin 300 (0.05%).
8	TMB Substrate	Tetramethyl-benzidine with citrate-phosphate buffer containing hydro-peroxide (H_2O_2): STORE IN THE DARK. Ready to use.
9	Stop Solution	1N sulfuric acid. Ready to use.
10	Adhesive Plate Sealer	
11	Instructions for Use	

■ Materials Needed, but Not Provided

1) Precision pipettes
2) Disposable pipette tips
3) Distilled/Deionized water
4) Wash bottle
5) Bichromatic spectrophotometer

■ Precautions

In order to obtain reproducible results, the following rules must be observed.

1) Store the components at 2~8 ℃ right after use. Do not reuse microwells or pour reagents back into their original bottles once dispensed.
2) Do not intermix components from kits with different batch numbers.
3) Do not use reagents after the expiry date.
4) Do not reuse containers and residues. Avoid contamination of each reagent with sample or other reagents.
5) Handle all reagents and samples as biohazardous materials.
6) Wear the gloves when you handle the potentially infectious materials. After handling, wash hands with sanitizers.
7) Keep all reagents away from skin and eyes. TMB Substrate and Stop Solution can cause irritation or burns to the skin and eyes. In case of an accident, rinse immediately with fresh cold water.
8) Dispose of containers and residues safely in accordance with national and local regulations.

■ Collection and Storage of Sample

1) Collect fresh colostrums from the sow.
2) Hemolyzed or contaminated samples may give erroneous results.
3) Samples should be stored at 2~8 ℃. For longer storage, freeze the samples at -20 ℃ or below. Avoid repeated freezing and thawing.

■ Preparation of Reagent

1) Unused microplate wells must be sealed with silica gel in enclosed sealing bag and stored at 2~8 ℃.
2) **Preparation of 101X Enzyme Conjugate**: Dilute the 101x Enzyme Conjugate by Conjugate Diluent (1:100). (e.g. Add 10 ㎕ of 101X Enzyme Conjugate to 1 ㎖ of Conjugate Diluent and mix thoroughly.)
3) **Preparation of 20X Washing Solution:** Dilute the 20x Washing Solution by distilled/deionized water (1:19). (e.g. Add 50 ㎖ of 20X Washing Solution to 950 ㎖ of distilled/deionized water and mix thoroughly.) In presence of undissolved crystals, re-suspend the solution by placing the vial at 37 ℃ for few minutes.
4) The storage and stability conditions of the prepared test reagents are as follows:

Reagents	State	Storage	Stability
Working Conjugate	Once prepared	Room temp.	Within 1 hour
		2 ~ 8 ℃	4 hours
Working Washing Solution	Once prepared	Room temp.	1 week

■ Procedure of the Test

1) Allow all reagents and samples to equilibrate at room temperature (18~25 ℃) for 30 minutes and shake them gently before use.
2) Prepare the Antigen Coated Microplate strip wells for Negative Control, Positive Control, and samples in duplicates.
3) Dispense 100 ㎕ of Sample Diluent into the strip wells.

4) Dispense each 10 ㎕ of Negative Control, Positive Control, and samples into the wells respectively. Cover the wells with an Adhesive Plate Sealer and shake the plate(s) gently. Shaking is very important to get the reproducible results.
5) Incubate the plate(s) at 37±1 ℃ for 60 minutes.
6) Aspirate all liquid from the wells and rinse the wells five times with 350 ㎕ of diluted washing solution. Remove any remaining washing solution by inverting the plate and blotting it against a clean paper towel.
7) Dispense 100 ㎕ of diluted enzyme conjugate to each well and cover the wells with an Adhesive Plate Sealer.
8) Incubate the wells for 30 minutes at 37±1 ℃.
9) Wash the wells as described above in the Step 6.
10) Dispense 100 ㎕ of TMB Substrate to each well and cover the wells with the Adhesive Plate Sealer.
11) Incubate the wells for 15 minutes at room temperature (18~25 ℃) in the dark.
12) Dispense 100 ㎕ of Stop Solution to each well. Mix by gentle shaking.
13) Read the absorbance values of the wells at 450 nm in a bichromatic spectrophotometer (with reference wavelength at 620 nm) right after from the end of the assay, within 30 minutes.

■ Interpretation of the Result

1) Test validation
① The mean OD of the Negative Control (NCx) ≤ 0.200.
② The mean OD of the Positive Control (PCx) ≥ 0.500.
③ If either of these values is out of range, the test result should be considered as invalid and the samples should be retested.

2) Interpretation of the Result
① Calculate the cut off value as following:
Cut off value = [0.35+ OD_{NCx}]

② Based on the cut off value, the results of samples are interpreted as follows:
- Positive Result: Mean OD of sample is above the cut off value.
- Negative Result: Mean OD of sample is less than the cut off value.

③ For example,
- OD_{NCx} : 0.078
- Cut off value: 0.35+ 0.078 = 0.428
  - Mean OD of sample: 0.186 → The sample result is interpreted as negative.
  - Mean OD of sample: 0.514 → The sample result is interpreted as positive.

■ Limitations and Interferences

1) For *in vitro* veterinary diagnostic use only.
2) Failure to add sample in the procedure could result in a falsely negative test. Repeat testing should be considered where there is a clinical suspicion of infection.
3) Other clinically available tests are required if questionable results are obtained. As other diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test. It is recommended the diagnosis decision is made by the veterinarian after all clinical and laboratory findings have been evaluated.

■ Stability and Storage

1) All reagents should be stored at 2~8 ℃. Do not freeze the test kit.
2) Shelf life is 12 months. Use all reagents before the expiry date on the kit.

■ Packaging Unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit
Antigen Coated Microplate	1 ea	5 ea
Negative Control	0.2 ml/vial x 1	1.0 ml/vial x 1
Positive Control	0.2 ml/vial x 1	1.0 ml/vial x 1
Sample Diluent	25 ml/bottle x 1	80 ml/bottle x 1
20X Washing Solution	50 ml/bottle x 1	250 ml/bottle x 1
101X Enzyme Conjugate	0.3 ml/bottle x 1	1.0 ml/bottle x 1
Conjugate Diluent	20 ml/bottle x 1	80 ml/bottle x 1
TMB Substrate	12 ml/bottle x 1	60 ml/bottle x 1
Stop Solution	15 ml/bottle x 1	80 ml/bottle x 1
Adhesive Plate Sealer	2 ea	10 ea
Instructions for Use	1 ea	1 ea

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