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 PED antigen on the well. For testing, ELISA plate is incubated with diluted samples and controls for 60 minutes at 37 ℃. During the first incubation, PED IgA Ab in sample binds onto the PED antigen coated on the well. After 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 to the complex is revealed by addition of substrate. The enzyme activity found on the well will thus be directly proportional to the PED IgA Ab in 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 reference wavelength at 620 nm.

The highly specifically selected PED antigens are used as capture material in the test. These enable the BIONOTE PED IgA Ab ELISA to identify PED IgA Ab in pig colostrums, with a high degree of accuracy.

■ Materials Provided

1	Antigen Coated Micro-Assay Plate	96 wells/plate, configured in twelve 1x8 strip. PED antigens coated on the wells.
2	Standard Negative Control	Colostrums and Proclin 300 (0.05%).
3	Standard Positive Control	Purified IgA to PED antigen in colostrums and Proclin 300 (0.05%).
4	Sample Diluent	Phosphate buffered saline and Proclin 300 (0.05%).
5	Washing Solution (20X concentrated)	PBS-Tween 20 and Proclin 300 (0.05%).
6	Enzyme Conjugate (101X concentrated)	Rabbit anti pig IgA-HRP, BSA and Proclin 300 (0.05%).
7	Enzyme 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	Stopping Solution	1N sulfuric acid. Ready to 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.

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

■ Collection and Storage of Sample

1) Collect fresh colostrums from the sow.
2) 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 Enzyme Conjugate (101X concentrated)**: Dilute the 101x Enzyme conjugate by Enzyme conjugate diluent (1:100). (e.g. Add 10 ㎕ of Enzyme conjugate to 1 ㎖ of Enzyme conjugate diluent and mix thoroughly.) Store at 2~8 ℃ or 18~25 ℃ after use.
3) **Preparation of Washing solution (20X concentrated)**: Dilute the 20x Washing solution by distilled/deionized water (1:19). (e.g. Add 50 ㎖ of Washing solution to 950 ㎖ of distilled/deionized water and mix thoroughly.) Store at 18~25 ℃ after use. In presence of undissolved crystals, re-suspend the solution by placing the vial at 37 ℃ for few minutes.

■ Procedure of the Test

1) Allow all reagents and samples to come to 18~25 ℃ for 30 minutes before use.
2) Prepare the antigen coated plate strip wells for standard negative control, standard positive control and samples in duplicates.
3) Dispense 100 ㎕ of sample diluent into the strip wells.
4) Dispense each 10 ㎕ of standard negative control, standard 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 Step 5.
10) Dispense 100 ㎕ of TMB substrate solution to each well and cover the wells with an adhesive plate sealer.
11) Incubate the wells for 15 minutes at room temperature (18~25 ℃) in the dark.
12) Dispense 100 ㎕ of stopping 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_{450} of the standard negative control (NCx) ≤ 0.200.
② The mean OD_{450} of the standard 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+NCx]

② Based on the cut off value, the result of samples are interpreted as follows:
-Positive Result: Mean OD_{450} of sample is above the cut off value.
-Negative Result: Mean OD_{450} of sample is less than the cut off value.

③ For example,
- NCx : 0.078
- Cut off value: 0.35+ 0.078 = 0.428
  - Mean OD_{450} of sample: 0.186 → The sample result is interpreted as negative.
  - Mean OD_{450} 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 specimen in the procedure could result in a falsely negative test. Repeat testing should be considered where there is 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 clinician after all clinical and laboratory findings have been evaluated.

■ Stability and Storage

1) All reagents should be stored at 2~8 ℃.
2) Shelf life of the product is 12 months after manufacturing.
3) Do not use after the stated expiry date.
4) Stability of once prepared reagents

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 weeks

■ Packaging Unit

Reagent \ Volume	96 Tests/Kit	480 Tests/Kit
Antigen Coated Micro-Assay Plate	1 ea	5 ea
Standard Negative Control	0.2 ml/vial x 1	1.0 ml/vial x 1
Standard 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
Washing Solution (20X concentrated)	50 ml/bottle x 1	250 ml/bottle x 1
Enzyme Conjugate (101X concentrated)	0.3 ml/bottle x 1	1.0 ml/bottle x 1
Enzyme Conjugate Diluent	20 ml/bottle x 1	80 ml/bottle x 1
TMB Substrate	12 ml/bottle x 1	60 ml/bottle x 1
Stopping 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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