SERELISA™ PEDV Ab Mono Indirect

zoetis

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EC REP

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PORCINE EPIDEMIC DIARRHEA VIRUS ANTIBODY TEST KIT

In-vitro diagnostic kit for the detection of Porcine Epidemic Diarrhea Virus (PEDV) antibodies in swine serum.

GENERAL INFORMATION AND INTENDED USES

Porcine Epidemic Diarrhea Virus (PEDV; Coronaviridae Alphacoronavirus) is a highly contagious coronavirus that has spread nationwide in the US after initial outbreaks in Europe and Asia. PEDV infects the intestine and can cause clinical diseases including diarrhea, vomiting, death, as well as substantial economic losses. Fecal shedding occurs before the onset of clinical signs.

SERELISA™ PEDV Ab Mono Indirect kit uses enzyme linked immunosorbent assay (ELISA) for the detection of PEDV antibodies in swine serum. The principle of the test is as follows: Recombinant PEDV Nucleocapsid (N) protein specific to circulating PEDV antibodies is directly coated onto the microwells. Serum and controls diluted in Sample Diluent are placed into the antigen-coated wells. PEDV antibody in the serum will form an antibody-antigen complex with the N proteins (antigen) bound to the microplate during a brief incubation period. After washing the microplate, a diluted horseradish peroxidase (HRP) conjugate mixture is added to each well. The antibody-antigen complex remaining from the previous step reacts with the conjugate. After a brief incubation period, the unbound conjugate is removed by a second wash step. Subsequently, ABTS Substrate, which contains a chromogen, is added to each well. A chromogenic color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. After incubation with the substrate, Stop Solution is added to each well to terminate the reaction and spectrophotometric values are recorded using an ELISA plate reader set at a single wavelength of 405 nm. Comparison of the assay results from unknown samples with those of known positive and negative controls provides the basis for determination of sample status in comparison to a sample/positive (S/P) cutoff.

KIT COMPOSITION AND CONSERVATION

This SERELISA™ PEDV Kit contains materials sufficient to test a maximum of 460 samples.

ITEM	REAGENT NATURE	VOLUME	RECONSTITUTION AND CONSERVATION
A	5 microplates containing 12 strips of 1 X 8 wells coated with PEDV N Protein Antigen	5 X 96 wells	Remaining wells may be used for up to 3 months after the pouch is first opened, provided the pouch is resealed and stored at 2 °C – 7 °C.
SD	Sample Diluent; preserved with Microcide III	1 X 110 mL	Ready to use (red color)
CONTROL -	40X Negative Control preserved with Microcide III	1 X 250 µL	Dilute to 1X in Sample Diluent upon addition to microplate. Use within 8 hours of dilution.
CONTROL +	40X Positive Control preserved with bromonitrodioxane	1 X 250 µL	Dilute to 1X in Sample Diluent upon addition to microplate. Use within 8 hours of dilution.
C	100X HRP-Conjugate Mixture; preserved with bromonitrodioxane	1 X 650 µL	Dilute to 1X in Conjugate Diluent. Use within 2 hours of dilution.
CD	Conjugate Diluent; preserved with bromonitrodioxane	1 X 65 mL	Ready to use (blue color)
W	20X Wash; preserved with Imidazole	1 X 200 mL	Dilute 1:20 in deionized or reverse osmosis water. Diluted Wash Solution can be stored at 18 °C - 28 °C. and used for up to 3 months following dilution.
ABTS	Substrate	1 X 100 mL	Ready to use
S	5X Stop (5 % SDS)	1 X 25 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted Stop Solution can be stored at 18 °C - 28 °C. and used for up to 3 months following dilution.

Note: All reagents provided in the kit should be stored at 2 °C – 7 °C. Reagents should not be frozen.

REAGENTS REQUIRED TO PERFORM 92 TESTS*

a) 1 PEDV Antigen coated microplate
b) 20 mL Sample Diluent
c) 10 µL 40X Negative Control
d) 10 µL 40X Positive Control
e) 110 µL 100X HRP Conjugate Mixture
f) 11 mL Conjugate Diluent
g) 20 mL 20X Wash
h) 11 mL Substrate
i) 2.5 mL 5X Stop

**Volumes required are applicable when 92 samples are run within one single plate.*

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

a) High precision multiple delivery pipetting devices (i.e., 1-20 µL). Measurement deviation must be ≤ 10 % for volumes ≤ 10 µL and ≤ 5 % for all other volumes)
b) 0.2 and 1.0 mL pipettes and tips
c) 8 or 12 channel pipettes and tips (or automated device)
d) 2 graduated cylinders (100 mL and 1000 mL)
e) Uncoated low binding 96 well microplates
f) Deionized or reverse osmosis water
g) Microplate washing apparatus
h) Microplate reader with **405 nm** filter

WARNINGS TO THE USERS OF REAGENTS AND ANTIGEN COATED MICROPLATES

- Handle all reagents and samples as biohazardous material. It is recommended to dispose reagents and contaminated material according to the applicable regulations.
- Wear suitable protective clothing.
- Take care not to contaminate any test reagents with samples or bacterial agents.
- If the humidity indicator of a microplate exhibits a pink color, the microplate should not be used.
- The best results are achieved by following the protocols described below, using good, safe laboratory techniques.
- Never add water to the microplates, conjugate, controls, or substrate.
- Do not use this kit after the expiration date.
- NEVER PIPETTE BY MOUTH. Harmful if swallowed.
- For veterinary use only.

Refer to the end of this insert for reagent hazard and precaution statements. Also reference the Safety Data Sheet for additional details.

SAMPLE COLLECTION

- Follow proper sample collection procedures.
- Harvest serum and store properly (up to four days at 2 °C – 7 °C, -20 °C for longer).
- Test only good quality serum (i.e., avoid bacterial contamination, heavy hemolysis or lipemia). When in doubt, obtain a better quality sample.

SAMPLE DILUTION PROCEDURE

Dilute serum samples using the Sample Diluent provided in a clean, uncoated 96 well microplate (Sample Dilution Microplate). Samples should be completely thawed and thoroughly mixed before diluting. Allow all reagents to come to room temperature (23 °C ± 5 °C) before starting!

STEP	UNITS	MATERIAL	LOCATION	FINAL DILUTION	NOTES
1)	195 µL	Sample Diluent	Each well	N/A	
2)	5 µL	Serum Sample	Add into all wells excluding the control wells (A1, A2, B1, B2)	1:40	Mix well. Discard pipette tips after each sample.
3)	5 µL	40X Negative Control	Into wells A1 and A2	1:40	
4)	5 µL	40X Positive Control	Into wells B1 and B2	1:40	

ALTERNATE SAMPLE AND CONTROL PREPARATION

A sample dilution plate is not required for small sample number testing. All samples can be diluted 1:40 with Sample Diluent in appropriate vials or microfuge tubes (mix well, vortexing is preferred) and added directly to the test wells to begin the first 30 minute incubation. Be sure to return any unused wells to the zip-pouch with desiccant for storage at 2 °C – 7°C.

PREPARATION OF 1X CONJUGATE, 1X WASH, AND 1X STOP SOLUTIONS

STEP	UNITS	MATERIAL	LOCATION	NOTES
1X CONJUGATE SOLUTION				
5)	10.89 mL	Conjugate Diluent	Clean tube or bottle	Mix well. 1:100 final dilution
6)	110 µL	100X Conjugate		
1X WASH SOLUTION				
7)	20 mL	20X Wash	Microplate washing bottle or apparatus	Mix well. 1:20 final dilution
8)	380 mL	Deionized or reverse osmosis water		
1X STOP SOLUTION				
9)	2.5 mL	5X Stop	Clean tube or bottle	Warm 5X Stop at room temperature or 37 °C and mix to dissolve any precipitates.
10)	10 mL	Deionized or reverse osmosis water		Mix well. 1:5 final dilution

ELISA TEST PROCEDURE

STEP	UNITS	MATERIAL	LOCATION	NOTES
a)	Remove the test microplate (or the required number of strips if whole microplate is not needed) from the protective bag.			
b)	100 µL	Diluted serum samples	Add into each test microplate well excluding control wells	Change pipette tips for each new sample
c)	100 µL	Negative Control	A1 and A2	Discard pipette tip
d)	100 µL	Positive Control	B1 and B2	Discard pipette tip
e)	Incubate microplate for 30 minutes (± 5 min) at 23 °C ± 5 °C.			

WASH PROCEDURE

STEP	UNITS	MATERIAL	LOCATION	NOTES
f)	Either manually or with an automated washer, discard or aspirate solution from all wells into an appropriate waste container.			Wash process is a critical step for an ELISA. Please follow steps f through j.
g)	300 µL	Diluted Wash solution (steps 7-8)	Each test well	
h)	Discard or aspirate solution from all wells into an appropriate waste container.			
i)	**Repeat wash procedure (steps g through h) two more times.**			
j)	Invert and blot onto absorbent pad to remove remaining liquid after final wash			

ADDITION OF 1X CONJUGATE, SUBSTRATE, AND 1X STOP SOLUTION

STEP	UNITS	MATERIAL	LOCATION	NOTES
k)	100 µL	1X Conjugate Solution (steps 5 - 6)	Each test well	Discard pipette tips.
l)	Incubate for 30 minutes (± 5 min) at 23 °C ± 5 °C.			
m)	Repeat the **WASH PROCEDURE** above (steps f to j).			
n)	100 µL	Substrate	Each test well	Discard pipette tips.
o)	Incubate for 15 minutes (± 1 min) at 23 °C ± 5 °C.			
p)	100 µL	1X Stop Solution (steps 9 - 10)	Each test well	Discard pipette tips.
q)	Gently tap to mix contents within the wells. Allow bubbles to dissipate and wipe the bottom of the microplate before reading. Read the microplate using an ELISA microplate reader set at a single wavelength of **405 nm**.			

RESULTS

AN ASSAY IS VALID WHEN THE CONTROLS GENERATE THE AVERAGE OPTICAL DENSITY (OD) VALUES LISTED BELOW:
Negative Control OD < 0.250
Positive Control OD ≥ 0.300
If either value is out of range, the test results should be considered invalid and the samples should be retested.

MANUAL PROCESSING OF DATA
a) Calculate the average Negative Control absorbance optical density (OD) using the values of A1 and A2. Calculate the average Positive Control OD using values obtained from wells B1 and B2. Record both averages.
b) Subtract the average Negative Control OD from the average Positive Control OD. The difference is the Corrected Positive Control.
c) Calculate a Sample to Positive (S/P) ratio by subtracting the average Negative Control OD from each sample OD and dividing the difference by the Corrected Positive Control. Use the following equation format:

$$\text{S/P} = \frac{\text{(SAMPLE OD) - (AVERAGE NEGATIVE CONTROL OD)}}{\text{CORRECTED POSITIVE CONTROL}}$$

EXAMPLE:
Example Positive Control ODs:
0.680 and 0.710
Average = (0.680 + 0.710) / 2 = 0.695

Corrected Positive Control:
(0.695) – (0.155) = 0.540

Example Negative Control ODs:
0.150 and 0.160
Average = (0.150 + 0.160) / 2 = 0.155

Example S/P value calculation:
OD of sample = 0.560
(0.560) – (0.155) / 0.540 = 0.750

INTERPRETATION OF RESULTS
SERELISA™ PEDV Ab Mono Indirect S/P values obtained for sera should be interpreted using the following ranges:

a) Negative: Serum samples with S/P ratios of < 0.190 are presumed negative for PEDV antibody.
b) Positive: Serum samples with S/P ratios of ≥ 0.190 are presumed positive for PEDV antibody.

NOTE: Individual diagnostic results should be interpreted with consideration of the history, clinical signs and assay results across the entire herd. Confirmatory or additional testing may be warranted.