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Porcine epidemic diarrhea virus (PEDV) Detection Kit

Product Number: DTL0839

Shipping and Storage

1. Store below 30℃. It is valid for 12 months.
2. Transport at normal temperature, not suggested over 14 days.
3. Opened but not completely used the all components should be stored at (-20±5)℃. It is recommended to separate in PCR tubes before refrigeration to avoid repeated freezing and thawing of all reagents next time. It is not recommended to repeat the freeze-thaw cycle more than 7 times.
4. Date of manufacture and term of validity: see the label.

Component

Component	48T
PEDV RT-PCR Master Mix	Lyophilized powder ×1 Bottle
Positive Control	100μL
Negative Control	1mL
Redissolved Diluent	1.5mL

Note: Do not mix reagents from different batches.

.The reaction system is lyophilized powder that contains all components required for fluorescence PCR, including Taq enzyme, reverse transcriptase, primers, probes, dNTPs, and Mg^{2+} etc.

Description

This kit uses a pair of porcine epidemic diarrhea virus-specific primers combined with a specific fluorescent probe to perform in vitro amplification and detection of porcine epidemic diarrhea virus RNA using one-step fluorescent RT-PCR technology, and is used for clinical etiological diagnosis of suspected infected samples.

Application

Porcine epidemic diarrhea (PED) is a highly contagious enteric disease caused by porcine epidemic diarrhea virus (PEDV), characterized by diarrhea, vomiting, dehydration and high mortality in suckling piglets. The disease can occur all year round. Sick pigs and latently infected pigs are the main sources of infection. The virus is transmitted through the fecal-oral route or the milk of infected sows. The disease is difficult to distinguish from porcine transmissible gastroenteritis, which has similar clinical symptoms. Fluorescent PCR technology facilitates the identification of these two pathogens.

This kit is suitable for detecting porcine epidemic diarrhea virus RNA in intestinal contents, mesenteric lymph nodes, excrement and other specimens, and is suitable for auxiliary diagnosis of porcine epidemic diarrhea virus infection.

Applicable instruments

Real-time fluorescence PCR instrument with FAM,VIC channels.

Specimen collection

1. Applicable sample type: Serum specimens.
2. Sample collection: Collect intestinal contents and mesenteric lymph nodes from autopsy pigs. Collect excrement from live pigs to be examined and place them in centrifuge tubes.
3. Sample storage and transportation: Excreta samples: Use 0.5g in a 1.5mL sterile centrifuge tube, add 1mL of physiological saline, vortex and oscillate, centrifuge at 8000rpm for 2min, and take 100μL of the supernatant in a 1.5mL sterile centrifuge tube. Tissue samples: Samples were taken from three different locations of mesenteric lymph nodes, and about 1g of the sample

was weighed, cut and mixed evenly by surgical scissors, and then 0.5g was weighed and ground in a grinder, and 1.5mL of normal saline was added to continue grinding. After homogenization, it was transferred to a 1.5mL sterilized centrifuge tube, and centrifuged at 8000rpm for 2min, and 100μL of supernatant was taken in a 1.5mL sterilized centrifuge tube.

4. Sample storage and transportation: The collection or processing sample should not exceed 24 hours under the conditions of 2℃ ~ 8℃. If long-term preservation is needed, it should be stored below -70°C, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the PEDV RT-PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 ~ 60 seconds until the liquid is clear and transparent. Subpackage it into PCR reaction tubes according to 20μL/ tube.

2. Nucleic acid extraction:

This kit is not included for Nucleic Acid(NA) extraction reagent.

Commercially available extraction kits that have been shown to generate highly purified RNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted RNA is not used immediately, it should be stored below -20℃. For long-term storage, it should be stored below -80℃ and avoid repeated freezing and thawing.

Note: The Negative Control and the Positive Control does not require nucleic acid extraction.

3. Add sample:

The correspond substances were added to that above PCR reaction tubes according to the following table:

Type	Add sample description
Testing Sample	Add 5μL of the extract prepared in step 2 to the reaction tube, and close the tube cover.
Negative Control/ Positive Control	Add 5μL of negative control and positive control to the reaction tube, and cover the tube tightly.

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be centrifuged for 5s on a palm centrifuge and then delivery to the nucleic acid amplification region. If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

4. PCR amplification:

Place the reaction tube in the automatic fluorescent PCR instrument, set the negative control, positive control, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding sample name.

Select FAM channel to detect PEDV RNA,select VIC channel to detect Internal control. Set the sample reaction system to 25μL.

(Note: For ABI series instruments, select 'None' under 'Quencher', and select 'None' as the dye to use as the passive reference.)

Recommended reaction program setting:

Step	Cycles	Temperature	Time	Collect fluorescence signal
1	1 cycle	50℃	10min	No
2	1 cycle	95℃	2min	No
3	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result analysis:

After the reaction is completed, the results are automatically saved.

The Start value, End value and Threshold value of the Baseline should be adjusted according to the analyzed image (the user can adjust it according to the actual situation, the Start value can be set at 3~15, the End value can be set at 5~20, the amplification curve of the negative control should be adjusted to be flat or below the threshold line).

Click Analyze for analysis, make the parameters meet the requirements in the following '6.Quality control', and then go to the Plate window to record the Ct value.

6. **Quality control**

Negative control: FAM and VIC detection channel has no obvious amplification curve.

Positive control: FAM and VIC detection channel has an obvious amplification curve, and the Ct value ≤32.00.

The above requirements must be met at the same time in the same experiment, otherwise this experiment is invalid and needs to be repeated.

Explanation of Test Result

1. Positive:The Ct value of the FAM channel is ⩽40.00, and the curve has an obvious exponential growth curve, and the VIC channel has or has no amplification curve.
2. Negative:The Ct value of the FAM channel is >40.00 or no Ct value, and the Ct value of the VIC channel is ⩽35.00.
3. If the amplification curve of the FAM channel of the test sample has no logarithmic growth phase and the Ct value of the VIC channel is >40.00, the test result of the sample is invalid and the cause should be found and eliminated. Collect the sample again and repeat the experiment.

Limitation

1. Sample detection results are related to sample collection, processing, transportation and preservation quality.
2. If cross-contamination is not controlled during the sample extraction process, false positive results will occur.
3. Contamination from the Positive Control or amplified products (amplicons) can lead to false positive results.
4. Genetic mutations or rearrangements in the target sequence may lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Degradation of reagent performance due to improper transportation, storage, or inaccurate preparation may lead to false negative results or inaccurate quantification.
7. The results of this test are for reference only. Clinical diagnosis should be based on a combination of clinical symptoms, other laboratory findings, and epidemiological data.

Performance Parameters

1. Minimum detection limit: The minimum detection limit of this reagent is 500 copies/mL.
2. Precision: The coefficient of variation (CV, %) of the Ct value of the pathogen detection channel is ⩽5.00%.
3. Compliance rate of negative/positive reference products: The compliance rate of negative reference products in enterprise reference is 100%, and the compliance rate of positive reference products is 100%.

Note

1. Please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps.
2. Before the test, please be familiar with and master the operation method and precautions of various instruments to be used, and carry out quality control for each experiment.
3. The reaction solution should be stored away from light.
4. Try to avoid bubbles in the reaction, and the tube cover needs to be tight.
5. Use disposable heads, disposable gloves and special work clothes in each district.
6. Sample processing, reagent preparation, and samples need to be performed in different areas to avoid cross -pollution.
7. After the experiment is completed, use 10% hypochlorite or 75% alcohol or ultraviolet light to treat the workbench and pipette.
8. All items in the kit should be treated as pollutants and processed in accordance with the "Biological Safety General of Microbiological Biomedical Laboratory".