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Porcine circovirus universal (PCV-U) Detection Kit

Product Number: DTL0802

Shipping and Storage

1. Store below 30°C. 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)°C. 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
PCV-U PCR Master Mix	Lyophilized powder ×1 Bottle
Positive Control	100μL
Negative Control	1mL
Redissolved Diluent	1.5mL

1. Do not mix reagents from different batches.
2. 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+}.

Description

This kit designs specific primers and probes for the conservative region of the porcine circovirus gene, uses fluorescent PCR technology to perform in vitro amplification and detection of the universal DNA of porcine circovirus, and is used for clinical etiological diagnosis of suspected infected animals.

Application

Porcine circovirus is a contagious, congenital tremor and multi-system dysfunction disease in weaned piglets caused by porcine circovirus. The virus has two serotypes, PCV1 and PCV2, and PCV1 is non-pathogenic. Weaned piglets infected with PCV2 have shortness of breath, difficulty, diarrhea, anemia, obvious lymphoid tissue lesions and progressive weight loss.

This kit is suitable for detecting porcine circovirus universal in samples such as lung, lymph node tissue, and whole blood, and is used for auxiliary diagnosis of porcine circovirus universal infection.

Applicable instruments

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

Specimen collection

1. Applicable sample type: Tissue samples (including porcine lymph nodes, spleen, lung, kidney and liver), serum, feces, cell culture.
2. Sample collection: Tissue sample: Collect the main organs (lymph nodes, spleen, lungs, kidneys and liver) of dead pigs (including aborted fetuses) or culled pigs and place the sample in sterilized 15mL centrifuge tubes and send it to the laboratory. Take 1g of thawed tissue, cut it into pieces, add 2mL PBS for grinding, prepare tissue homogenate, centrifuge at 8000 r/min for 5 min, and take the supernatant for subsequent nucleic acid extraction; Serum sample: Use a sterile syringe to draw no less than 5mL of venous blood from the tested pig, place it in a sterile centrifuge tube, place it at an angle at room temperature or 37℃ to allow natural coagulation for 20 min to 30 min, centrifuge it at 2000 r/min to 3000 r/min for 10 min, and aspirate the

supernatant into a new centrifuge tube for direct nucleic acid extraction; Fecal sample: Take 1g of fresh pig feces, put it into a 15mL sterile centrifuge tube, add 5mL PBS, and mix well. Centrifuge at 3000 r/min for 10 min, take the supernatant, and transfer it to a 1.5mL centrifuge tube for subsequent nucleic acid extraction.

3. 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℃, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the PCV-U PCR Master Mix, open each 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 DNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted DNA 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 separately, and cover the tube tightly.

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be mixed well and 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 PCV-U DNA,select VIC channel to detect Internal Control. Set the Reaction Volume per Well 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	95℃	2min	No
2	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,VIC detection channels have no amplification curves.

Positive control: FAM,VIC detection channels have obvious amplification curves, and the Ct value of each channel ≤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. The FAM channel is the detection result of porcine circovirus, and the VIC channel is the detection result of porcine endogenous internal control.
2. PCV-U Positive:Ct value ≤40.00 and the curve has a clear index growth curve.
3. PCV-U Negative:Ct value >40.00 or no Ct value.
4. Suspicious samples: If the sample test result is 40.00<Ct value≤45.00, it is recommended to repeat the test. If the test channel is still 40.00<Ct value≤45.00 and the curve has an obvious growth curve, it is judged as positive, otherwise it is negative.

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. Positive control and leakage of amplification products can lead to false positive results.
4. The genetic mutations and reorganizations during epidemics can lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Reagent transportation, improper preservation, or inaccurate reagent preparation reagent detection performance decreases, and the results of false negative or quantitative detection occur.
7. The results of this test are for reference only. If the diagnosis must be confirmed, please combine clinical symptoms and other test methods.

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% hypochloride 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".