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Avian encephalomyelitis virus (AEV) Detection Kit

Product Number: DTL0897

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
AEV RT-PCR Master Mix	Lyophilized powder ×1 Bottle
Positive Control	100μL
Negative Control	Lyophilized powder ×1 Tube
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+}.
3. Add 100μL Redissolved Diluent to the Negative Control, mix well, and use a palm centrifuge to centrifuge briefly before use.

Description

This kit uses a pair of avian encephalomyelitis virus-specific primers combined with a specific fluorescent probe to perform in vitro amplification and detection of avian encephalomyelitis virus RNA using a one-step fluorescent RT-PCR technique for clinical etiological diagnosis of suspected infected samples.

Application

This kit is suitable for detecting avian encephalomyelitis virus RNA in specimens such as brain tissue, pancreas, glandular stomach, and whole blood, and is suitable for auxiliary diagnosis of avian encephalomyelitis virus infection.

Applicable instruments

Real-time fluorescence PCR instrument with FAM,CY5 channel.

Specimen collection

1. Applicable sample type: 2g of brain tissue, pancreas and glandular stomach of dead or culled poultry. For live poultry to be tested, use a syringe to collect 5mL of blood into an EDTA-2Na anticoagulant tube.
2. Sample collection: Blood sample: After the whole blood sample is coagulated, 100μL of serum is taken and placed in a 1.5mL centrifuge tube for later use; Tissue sample: Weigh about 1g of sample from different locations of each tissue, chop it with surgical scissors and mix it, then take 0.5g and grind it in a grinder, add 1.5mL of physiological saline and continue grinding. After homogenization, transfer it to a 1.5mL sterile centrifuge tube, centrifuge it at 8000rpm for 2min, take 100μL of the supernatant and put it in a 1.5mL sterile centrifuge tube for later use.
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 AEV RT-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 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.The Negative Control needs to be redissolved with 100μL of Redissolved Diluent and mixed well before use.

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 mix evenly for 5 seconds with a vortex shaker,centrifuged for 5 seconds 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 AEV RNA,select CY5 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 detection channel have no amplification curves, CY5 channel amplification curve has an obvious exponential growth phase, and the Ct value is ≤32.00.

Positive control: FAM and CY5 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. The FAM fluorescence channel is the detection result of avian encephalomyelitis virus, and the CY5 channel is the detection result of 18S Internal control gene.
2. Positive:Ct value ≤40.00 and the curve has a clear index growth curve.
3. Negative:The FAM channel of sample test results have no Ct value and no specific amplification curve,and the CY5 channel is ≤35.00.
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% 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".