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Avian Pasteurella multocida (PM) Detection Kit

Product Number: DTL0881

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 BA PCR Master Mix 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. Storage time should not exceed 21 days.
4. Date of manufacture and term of validity: see the label.

Component

Component	48T
BA PCR Master Mix	Lyophilized powder ×1 Bottle
Positive Control	Lyophilized powder ×1 Bottle
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+}.
3. Add 100μL Redissolved Diluent to the Positive Control, mix well, and use a palm centrifuge to centrifuge briefly before use.

Description

This kit utilizes TaqMan probe-based real-time fluorescence PCR technology, employing a pair of Bacillus anthracis-specific primers and a specific probe. It uses fluorescence PCR technology to amplify and detect Bacillus anthracis DNA in vitro, and is intended for the etiological diagnosis of suspected infected individuals. The kit is provided with an Internal Control, which can monitor whether there is PCR inhibitor in the sample to be tested by detecting whether the internal control is normal or not, so as to avoid false negative PCR.

Application

Anthrax is a zoonotic disease caused by infection with Bacillus anthracis (BA). BA forms spores in extreme environments and can persist stably in nature for extended periods. Due to its stability and pathogenicity, BA can easily trigger public health emergencies. Current BA detection methods are mainly based on pathogen culture, staining and microscopic examination, and serological testing, but these methods are not suitable for early and rapid detection. Fluorescence-PCR technology, based on nucleic acid detection, offers high sensitivity, simple operation, and good specificity, allowing for rapid detection of the anthrax pathogen and timely control of anthrax outbreaks.

This kit is suitable for detecting Bacillus anthracis in samples such as whole blood, skin lesions, feces and vomit, and cerebrospinal fluid, and is used for the etiological diagnosis of suspected infectious materials in clinical practice.

Applicable instruments

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

Specimen collection

1. Applicable sample type: Whole blood, skin lesions, stool and vomit, and cerebrospinal fluid samples.
2. Sample collection: Whole blood sample: Collect 5mL of blood using a syringe into an EDTA-2Na anticoagulant tube, and then

use it directly for nucleic acid extraction; Skin lesions sample: During the vesicular stage, use two sterile cotton swabs to collect fluid from the blisters. During the ulcer stage, use two sterile cotton swabs moistened with saline solution to swab the lesions. During the scab stage, repeatedly swab the scabs with two sterile cotton swabs moistened with saline solution; Stool and vomit: For suspected cases exhibiting gastrointestinal symptoms, collect stool or vomit specimens, paying particular attention to selecting portions mixed with blood, and place them in sterile containers.

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°C, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the BA 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 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. The Positive 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 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. RT-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 BA DNA, select VIC channel to detect the 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 and VIC detection channels have no amplification curves.

Positive control: FAM and VIC detection channels have obvious amplification curves, and the Ct value of each channel≤35.00.

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: If the Ct value of the detection channel is ≤40.00, and the curve has an obvious exponential growth curve, then the virus type corresponding to the channel is positive, and there is no IC requirement.
2. Negative: If the Ct value of the sample test result is >40.00 or there is no Ct value, and the Ct value of the IC is ≤40.00, then the virus type corresponding to the channel is negative.
3. If the FAM channel amplification curve of the test sample has no logarithmic growth phase and the VIC channel Ct value is >40.00, the test result of the sample is invalid and the cause should be found and eliminated. Collect samples 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% 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".