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Bacillus anthracis (BA) Detection Kit

Product Number: DTL1106

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
PM PCR Master Mix	Lyophilized powder ×1 Bottle
PM quantitative standard substance 1	Lyophilized powder ×1 Bottle
PM quantitative standard substance 2	Lyophilized powder ×1 Bottle
PM quantitative standard substance 3	Lyophilized powder ×1 Bottle
PM quantitative standard substance 4	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+}.
3. Add 100μL Redissolved Diluent to the PM quantitative standard substances 1~4 each, mix well, and use a palm centrifuge to centrifuge briefly before use.

Description

This kit designs primers and probes for the specific nucleic acid sequence of avian Pasteurella multocida, uses fluorescent PCR technology to perform in vitro amplification and detection of avian Pasteurella multocida DNA, and is used for clinical etiological diagnosis of suspected infected samples.

Application

This kit is suitable for the detection of avian Pasteurella multocida DNA in the head, liver, and spleen tissues of avian Pasteurella multocida disease, and is suitable for the auxiliary diagnosis of avian Pasteurella multocida infection.

Applicable instruments

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

Specimen collection

1. Applicable sample type:For animals that died of disease or were culled, the head, liver, and spleen tissues of suspected diseased poultry were collected aseptically.
2. Sample collection: Aseptically collect 1.0g to 5.0g of head, liver, and spleen tissues from suspected avian Pasteurella multocida, add PBS at 10% (g/mL), grind and homogenize, and store at -20℃ 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 PM 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 19μL/ tube.

Take out the Exogenous Internal Control and open the tube cap,add 100μL of Redissolved Diluent,mix evenly for 15 seconds with a vortex shaker, centrifuge for 3 seconds with a palm centrifuge, and then subpackage it into PCR reaction tubes according to 1μL/ tube.

The total test reaction system mixed well between the PM PCR Master Mix and Exogenous Internal Control is 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 PM quantitative standard substances 1~4 needs to be redissolved with 100μL of Redissolved Diluent and mixed well before use separately.

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
PM quantitative standard substances 1,2,3,4	Add 5μL of PM quantitative standard substances 1,2,3,4 respectively, to the PCR reaction tube, 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 avian Pasteurella multocida 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 detection channel has no obvious amplification curve,in the Reports interface, the Ct column displays 'Undet'.

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

PM quantitative standard substances 1~4:FAM channel has obvious amplification curve, and the standard curve correlation coefficient $|r| \geq 0.98$.

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. If the Ct value of the VIC channel of the test sample is >35.00, the test result of the sample is invalid, and the cause should be found and eliminated. Samples need to be recollected and the experiment repeated.
2. For samples with measured value $<5.00\times10^2$ copies/mL or Ct value was 'Undet', and the Internal Control detection is positive and Ct value ≤32.00, it was reported as lower than the detection limit of the kit. If the Internal Control is abnormal, the test result of this sample is invalid, and the test is repeated for this sample.
3. For samples with measured values between 5.00×10^2 copies/mL~1.00×10^8 copies/mL, report the corresponding determination results.
4. For samples with measured value >1.00×108 copies/mL, the Internal Control detection is positive and Ct value ≤32.00, It is recommended to re-measure after diluting with 1×TE, calibrate the results according to the dilution factor, report the calibrated value, and indicate "reportable concentration",and the measurement results are for reference only.

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. Limit of Detection: The minimum detection limit of this reagent is 500 copies/mL.
2. Linear range: 5.00×10^2 copies/mL~1.00×10^8 copies/mL.
3. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) value of detected concentration logarithm is ≤5.00%.
4. 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".