

Diagnostic Kit for Pseudomonas Aeruginosa (PA) DNA

Product Number: DTL0605

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
PA 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 contains all components required for fluorescence PCR, including Taq enzyme, primers, probes, dNTPs, and Mg²⁺.

Description

This kit uses a pair of specific primers and one specific probe for Pseudomonas aeruginosa. It amplifies and detects the nucleic acid of the pathogen in vitro by fluorescent PCR, for the etiological diagnosis of clinically suspected infectious samples. 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

This kit is used to detect Pseudomonas aeruginosa in samples such as wound exudate, suspected contaminated water, food and cosmetics, serving as an auxiliary tool for the diagnosis of Pseudomonas aeruginosa infection.

Applicable instruments

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

Specimen collection

1. Applicable sample type: Suitable for wound exudate, suspected contaminated food and cosmetics sample.
2. Sample collection: Suspected contaminated food or cosmetics sample: Take 1 gram of suspected contaminated food or cosmetics. Cut and mix the food thoroughly with surgical scissors, then weigh 0.05g of the sample into a grinder. Add 1.5mL of normal saline and continue grinding until homogenized. Transfer the homogenate into a sterile 1.5mL centrifuge tube, centrifuge at 8000 rpm for 2 minutes. Pipette 100µL of the supernatant into another sterile 1.5mL centrifuge tube for nucleic acid extraction; Wound exudate sample: Pipette 100µL of wound exudate directly into a sterile 1.5mL centrifuge tube for nucleic acid extraction.
3. Sample storage and transportation: The collection or processing sample should not exceed 24 hours under the conditions of 2°C ~ 8°C. 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 PA 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°C. For long-term storage, it should be stored below -80°C 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 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 PA 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°C	2min	No
2	45 cycles	95°C	15sec	No
		60°C	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 channels has no obvious amplification curve.

Positive Control: FAM, VIC channels all has an obvious amplification curve, and the Ct value ≤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 target channel 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 target channel 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. 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. Limit of Detection: The minimum detection limit of this reagent is 500 copies/mL.
2. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) of the Ct value of the pathogen detection channel is $\leq 5.00\%$.
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".