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Vibrio alginolyticus Nucleic Acid Real-Time Fluorescent PCR Detection Kit

Product Number: DTK561

Shipping and Storage

1. Transportation: The reagent kit must be transported under frozen conditions.
2. Storage: Store at -20℃ and avoid repeated freezing and thawing. The freeze-thaw cycle of the reagent kit shall not exceed 7 times.
3. Validity period: 12 months, please use within the validity period.

Component

Component	50T
Nucleic acid amplification reaction mixture	900μL
Enzyme mixture	100μL
Positive control of Vibrio alginolyticus	100μL
Negative control	1000μL

Note: Different batches of reagents cannot be mixed.

Description

This kit uses real-time fluorescence PCR technology and is suitable for nucleic acid detection of Vibrio alginolyticus extracted from bacterial cultures, pure cultures, or single colonies obtained from clinical samples, food samples, and preserved bacterial strains. Each reaction system contains specific primers and fluorescent probes for detecting Vibrio alginolyticus genes. By collecting the fluorescent signal generated by PCR amplification, qualitative detection of Vibrio alginolyticus nucleic acid can be quickly completed.

Application

This kit is suitable for qualitative detection of Vibrio alginolyticus nucleic acid extracted from bacterial cultures, pure cultures, or single colonies obtained from clinical samples, food samples, and preserved bacterial strains. The experimental results only provide reference for basic research and are not used as clinical diagnostic basis.

Vibrio alginolyticus lives in seawater or freshwater, and is more common in summer or autumn, especially in coastal areas. Some can cause sepsis, which should be taken seriously.

Applicable instruments

The fully automatic fluorescence PCR detector that has undergone multi-channel calibration needs to include a FAM detection channel.

Specimen collection

DNA samples extracted from clinical samples, food samples, and preserved bacterial strains through cultivation, pure cultures, or single colonies can all be tested using this kit.

Protocol

1. Sample Preparation

Perform nucleic acid extraction using the bacterial DNA extraction reagent provided with this kit. The extracted DNA can be directly used for detection. If the sample is not tested immediately after extraction, it can also be stored at -20℃ or -70℃ for

later use. Repeated freezing and thawing should be avoided.

2. Preparation of reaction system

2.1. System configuration

Take out the reagent from the kit and melt it at room temperature. Wait for the reagent to completely thaw, invert and mix well, and then centrifuge immediately. If the number of samples to be tested is n (n=number of samples+positive control+negative control), prepare the system according to n+1 reactions. The reaction system is prepared as shown in the following table:

reagent	Quantity of 1 reaction system	Quantity of n+1 reaction systems
Nucleic acid amplification reaction mixture	18μL	18μL×(n+1)
Enzyme mixture	2μL	2μL×(n+1)

2.2. System packaging:

After mixing and centrifuging the above reaction solution, package 20μL per tube into PCR tubes suitable for fluorescence PCR equipment.

2.3. Sample addition:

Take 5μL of DNA samples extracted in step 1 and add them to the pre packaged PCR reaction tubes. Tighten the tube cap, gently mix, and centrifuge immediately before moving to the amplification zone. The total reaction volume is 25μL. Add 5μL of negative control to the negative control reaction tube and 5μL of corresponding template to the positive control reaction tube.

3. Fluorescence PCR cycle condition setting

step	Cycles	Temperature	Time	
1	1 cycle	95℃	30min	
2	40 cycles	95℃	5sec	
		60℃	30sec	Collect fluorescence

Other instruments, such as ABI7500, set the fluorescence collection time to 31 seconds and have no effect on the results.

Detection settings: "Reporter Dye" is set to FAM, corresponding to the detection of Vibrio alginolyticus nucleic acid, and "Quencher Dye" is set to None. For ABI series instruments, please note to set "Passive Reference" to None.

4. Threshold setting

The threshold setting principle is to use the highest point of the fluorescence signal that just exceeds the normal negative control as the threshold line, or adjust it according to the instrument noise situation.

5. Result analysis and judgment

5.1. If the sample has S-type amplification in the FAM channel and the Ct value is ≤ 35, it is determined to be positive for Vibrio alginolyticus nucleic acid;

5.2. If the sample has S-type amplification in the FAM channel and 35<Ct value ≤ 40, it is determined as an uncertain sample and requires re extraction of nucleic acid for testing; If the FAM channel of the retested sample still has S-type amplification and the Ct value is ≤ 40, it is judged as positive for Vibrio alginolyticus nucleic acid, otherwise it is judged as negative;

5.3. If there is no obvious S-type amplification curve in the FAM channel of the sample, but Ct values are reported, it is still judged as negative for Vibrio alginolyticus nucleic acid.

Quality control standards

The negative control had no amplification curve, and the positive control had an S-shaped amplification curve in the FAM detection channel, indicating the validity of the experiment. Otherwise, the experimental results will be deemed invalid.

Limitations of detection methods

The target sequence detected by this kit is the conserved region of the Vibrio alginolyticus gene, which is highly conserved. But if bacteria undergo genetic mutations at the target sequence, false negative results may occur, that is, missed detection; Meanwhile,

the quality of sample collection, processing, transportation, and preservation all have an impact on the test results.

Product performance indicators

1. Minimum detection limit: 5×10^2CFU/mL.
2. Linear range: 5×10^2~2×10^{10} CFU/mL.
3. Cross reactivity: There is no cross reactivity with other pathogens that may cross with Vibrio alginolyticus, such as Escherichia coli, Vibrio parahaemolyticus, Staphylococcus aureus, Salmonella, Shigella, Clostridium botulinum, Cronobacter, Listeria monocytogenes, Yersinia enterocolitica, Aeromonas, Bacillus cereus, Klebsiella, Campylobacter, Clostridium perfringens, Norovirus, rotavirus, hepatitis A virus, hepatitis E virus, adenovirus, Aspergillus flavus, and Fusarium.
4. Precision: The coefficient of variation of the precision reference sample is less than 5%.

Note

1. Please read the instructions of this reagent kit carefully before the experiment and strictly follow the operating steps.
2. The components inside the container should be thoroughly melted and mixed before use, and then subjected to a brief high-speed centrifugation before use.
3. The reagent kit must be stored away from light to prevent the decay of fluorescent substances. The centrifuge tubes and Tip heads used should be sterilized under high pressure and free of DNase and RNase.
4. The entire operation process and the software and hardware facilities of the PCR laboratory should comply with the requirements of regulations such as the "Management Measures for Clinical Gene Amplification Testing Laboratories in Medical Institutions" and the "Guidelines for the Work of Clinical Gene Amplification Testing Laboratories in Medical Institutions" issued by the Ministry of Health. Properly handle the waste and amplification products generated during the experimental process to prevent cross contamination.
5. The reference standard for this test kit is: SN/T 1870-2016 Detection Method for Foodborne Pathogens in Exported Foods - Real time Fluorescent PCR Method
6. This product is for scientific research only, and the test results are for reference only. If a diagnosis is required, please combine clinical symptoms and other testing methods.