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# Vibrio vulnificus Nucleic Acid Detection Kit (Fluorescent PCR

# Method)

### Product Number: DTK533

#### **Shipping and Storage**

- 1. Transportation: The reagent kit must be transported under frozen conditions.
- 2. Storage: Store at -20°C 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 solution	100µL
Positive control of Vibrio vulnificus	100µL
Negative quality control product	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 vulnificus 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 vulnificus genes. By collecting the fluorescent signal generated by PCR amplification, qualitative detection of Vibrio vulnificus nucleic acid can be quickly completed.

#### Application

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

Vibrio vulnificus is a bacterium that inhabits the ocean. Infection with this bacterium can lead to wound infections and primary sepsis. If the wound comes into contact with seawater, shells, or fish, it is also possible to be infected with the bacteria.

#### **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 obtained 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°C or -70°C for later use. Repeated freezing and thawing should be avoided.

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### 2. Preparation of reaction system

#### 2.1. System preparation:

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 table below:

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

#### 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  $5\mu$ L of DNA samples extracted in step 1 and add them to the pre packaged PCR reaction tubes. Tighten the tube cap, gently mix and shake, centrifuge instantly, and move to the amplification zone. The total reaction volume is  $25\mu$ L. Add  $5\mu$ L of negative control to the negative control reaction tube and  $5\mu$ L of corresponding template to the positive control reaction tube.

#### 3. Fluorescence PCR cycle condition setting

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

Detection settings: "Reporter Dye" is set to FAM, corresponding to the detection of Vibrio vulnificus 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 vulnificus nucleic acid;
- 5.2. If the sample has S-type amplification in the FAM channel and 35<Ct value  $\leq 40$ , 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  $\leq 40$ , it is judged as positive for Vibrio vulnificus 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 vulnificus 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 vulnificus gene, which is highly conserved and stable. 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.

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# **Product performance indicators**

- 1. Minimum detection limit:  $5 \times 10_2$ CFU/mL.
- 2. Linear range:  $5 \times 10_2 \sim 2 \times 10_{10}$  CFU/mL.
- 3. Cross reactivity: There is no cross reactivity with other pathogens that may cross with Vibrio vulnificus, 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. Before the experiment, please carefully read the instructions of this reagent kit and strictly follow the operating steps.
- 2. Each component in the reagent kit should be thoroughly melted and mixed before use, and then subjected to high-speed and brief 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. 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.