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Neisseria meningitidis (A, B, C) Serogroups Triple Real-Time Fluorescent PCR Detection Kit

Product Number: DTK558

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
MP328Z - Positive control	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 detecting Neisseria meningitidis (A, B, C) serogroup nucleic acid extracted from clinical samples such as cerebrospinal fluid and blood. Each reaction system contains specific primers and fluorescent probes for detecting the serogroup genes of Neisseria meningitidis (A, B, C). By collecting the fluorescent signal generated by PCR amplification, qualitative detection of Neisseria meningitidis (A, B, C) serogroup nucleic acid can be quickly completed.

Application

This kit is suitable for qualitative detection of Neisseria meningitidis (A, B, C) serogroup nucleic acid extracted from clinical samples such as cerebrospinal fluid and blood The experimental results only provide reference for basic research and are not used as clinical diagnostic basis.

Neisseria meningitidis is the pathogen responsible for epidemic cerebrospinal meningitis (meningitis). According to the different antigenicity of its capsule polysaccharides, Neisseria meningitidis can be classified into at least 13 serum groups.

Specimen collection

DNA samples extracted from clinical samples such as cerebrospinal fluid and blood can be tested using this kit.

Protocol

1. Sample preparation

According to the commercialized bacterial DNA extraction reagent for nucleic acid extraction, we recommend using our company's extraction kit for extraction. The extracted DNA can be directly used for testing. If the sample is not tested immediately after extraction, it can also be stored at -20℃ or -70℃ for future use, and repeated freezing and thawing should be avoided.

2. Preparation of Sample DNA

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 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 shake, centrifuge instantly, and move 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

Process	Number of cycles	Temperature	Time	
1	1 cycle	95℃	30sec	
2	40 cycles	95℃	5sec	
		60℃	30sec	Collect fluorescence

Detection settings: "Reporter Dye" is set to FAM, VIC (HEX), and CY5 respectively, and the corresponding channels for each target are detailed in the result interpretation. Quencher Dyes are all None. For ABI series instruments, please set the "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.

Quality control standards

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

Result analysis and judgment

a) If the sample has S-type amplification in the detection channel and the Ct value is ≤ 35, determine according to the fluorescence channel corresponding to the detection target according to the following table; b) If the sample has S-type amplification in the detection channel and 35<Ct value ≤ 40, it is determined as an uncertain sample and requires re extraction of nucleic acid for testing; If the retested sample still has S-type amplification in the detection channel and the Ct value is ≤ 40, the fluorescence channel corresponding to the detection target shall be judged according to the following table. Otherwise, it shall be judged as negative; c) If there is no obvious S-shaped amplification curve in the detection channel of the sample, but Ct value is reported, it is judged as negative.

Fluorescence channel (detection target)	FAM channel (Serum Group A)	VIC (HEX channel) (B serum group)	CY5 channel (Serum Group C)
Neisseria meningitidis serogroup A	+	−	−
Neisseria meningitidis serogroup B	−	+	−
Neisseria meningitidis serogroup C	−	−	+

Limitations of detection methods

The target sequence detected by this kit is the conserved region of the Neisseria meningitidis 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: 5×10^2 CFU/mL.
2. Linear range: 5×10^2 ~2×10^{10} CFU/mL.
3. Cross reactivity: No cross reactivity was observed against other pathogens that may cross with Neisseria meningitidis, such as Japanese encephalitis virus, mumps virus, enterovirus, coxsackievirus, herpes simplex virus, poliovirus, measles virus, respiratory syncytial virus, human cytomegalovirus, EB virus, Streptococcus pneumoniae, Listeria monocytogenes, Streptococcus agalactiae, Micrococcus, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecium, coagulase negative Staphylococcus, Cryptococcus neoformans, hemolytic Streptococcus suis.
4. Precision: The coefficient of variation of the reference standard for detecting precision 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.