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Neisseria meningitidis Serogroup Y Probe-Based Quantitative PCR Kit

Product Number: DTK557

Shipping and Storage

-20℃±5℃, store and transport in the dark, with no more than 5 freeze-thaw cycles, valid for 12 months.

Component

Component	50T
Neisseria meningitidis serogroup Y primer mixture	100μL
qPCR amplification reaction solution	900μL
Neisseria meningitidis serogroup Y positive control	100μL
Neisseria meningitidis serogroup Y negative control	100μL

Description

This kit is designed with specific primers and probes targeting the highly conserved regions of the Neisseria meningitidis serogroup Y genome. In the presence of Neisseria meningitidis serogroup Y genomic template in the reaction system, the PCR reaction proceeds and releases fluorescent signals. The real-time monitoring and output of the corresponding channel signals during the PCR process are achieved using a quantitative PCR instrument, enabling qualitative analysis of the test results.

Application

This kit is suitable for the detection of Neisseria meningitidis serogroup Y. Epidemic meningococcal meningitis (EMM) is caused by Neisseria meningitidis, and its classification is primarily based on differences in capsular polysaccharide antigens, divided into serogroups (such as A, B, C, W, X, Y, etc.). The pathogenicity, endemic regions, and vaccination strategies vary among different serogroups. It can be used as an auxiliary diagnostic tool for Neisseria meningitidis serogroup Y infections but is not intended for definitive diagnosis.

Applicable instruments

ABI series, Bio Rad series, Roche LC480, QIAGEN Rotor Gene series, Tianlong Gentier-96E/96R series, Yarui MA6000, Hongshi SLAN-96S/96P and other multi-channel real-time fluorescence quantitative PCR instruments.

Specimen collection

1. Sample selection: Neisseria meningitidis infection can cause epidemic cerebrospinal meningitis (meningitis), mainly transmitted through respiratory droplets. Common samples include cerebrospinal fluid, blood, nasopharyngeal swabs, etc. Appropriate samples should be selected for testing based on specific clinical conditions during collection.
2. Aseptic operation: When collecting samples, attention should be paid to aseptic operation to avoid external bacterial contamination.
3. Storage: Samples should be stored at 2-8℃ for no more than 72 hours, and can be stored for a long time below -70℃, but repeated freeze-thaw cycles should be avoided (up to 5 freeze-thaw cycles).

Protocol

1. **Sample processing (sample processing area)**

   We recommend using our company's commercial reagent kit to extract DNA/RNA, strictly following the instructions of the corresponding reagent kit, and avoiding contamination during the sample nucleic acid extraction process.

2. Reagent Preparation (Reagent Preparation Area)

Based on the total number of samples to be tested, the required number of PCR reaction tubes is N (N=number of samples+1 negative control tube+1 positive control tube); For every 10 samples, an additional 1 sample is prepared. The preparation of each test reaction system is shown in the following table:

Component	Volume
Neisseria meningitidis serogroup Y primer mixture	2μL
qPCR amplification reaction solution	18μL
Total volume	20μL

3. Sample addition (sample processing area)

Take 5μL of DNA/RNA, positive control sample, and negative control sample extracted in step 1, add them to the corresponding reaction tubes, cover the tubes, mix well, and briefly centrifuge.

4. PCR amplification

4.1. Place the reaction tube to be tested in the reaction tank of the fluorescence quantitative PCR instrument;

4.2. Set the channel and sample information, set the reaction system to 25μL; select the fluorescence channel: FAM for the detection channel (Reporter Dye) and NONE for the quenching channel (Quencher Dye), do not select ROX reference fluorescence.

4.3. Recommended loop parameter settings:

Step	Cycles	Temperature	Time
Pre denaturation	1 cycle	95℃	2min
Denaturation	40 cycles	95℃	10sec
Annealing/extension/fluorescence detection*		55℃	30sec

Note: When setting up the ABI series fluorescence PCR instrument, ROX calibration is not selected, and the quenching group is set to None.

5. Result analysis and judgment

5.1. Experimental effectiveness assessment

Positive control: There is a typical S-shaped amplification curve or Ct value ≤ 35.

Negative control: Ct value>40 or no Ct value, linear or slightly diagonal, no exponential growth period.

The above requirements must be met simultaneously in the same experiment, otherwise this experiment is invalid.

5.2. Sample result determination

Positive: The amplification curve shows an S-shape, and the Ct value of the detection channel is ≤ 35.0, with a significant exponential growth curve;

Suspicious: The amplification curve shows an S-shape and the detection channel has a Ct value of 35.0<≤ 40. It is recommended to repeat the test. If the detection channel still has a Ct value of 35.0<≤ 40 and the curve shows a significant growth curve, it is judged as positive. Otherwise, it is judged as negative;

Negative: The Ct value of the specimen test result is greater than 40 or there is no Ct value.

Limitations of detection methods

1. When the concentration of the tested nucleic acid in the test sample is lower than the minimum detection limit of this kit, false negative results may occur.
2. Improper handling of the collected, transported, stored, and processed samples can easily lead to DNA/RNA degradation and false negative results.
3. Cross contamination of samples during collection, transportation, storage, and processing can easily lead to false positive results.

Performance indicators of reagent kit

1. The minimum detection limit of the product is 200copies/mL;
2. Linear detection range: 200~1 × 10^{10} copies/mL;

3. Precision: The coefficient of variation (CV) within and between batches of the precision reference sample is ≤ 5%;
4. Strong specificity: There is no cross reactivity with other pathogens that have the same infection site or similar infection symptoms.

Note

1. All operations should be strictly carried out according to the instructions, and PCR operations should be strictly divided into zones at each stage to avoid cross contamination.
2. To avoid DNA/RNA degradation, the sample processing should be operated under 0-4℃ conditions, and immediately tested on the machine after the experiment is completed; The instruments and consumables used in the sample processing should be treated with ribonucleases.
3. Negative and positive controls should be set up for each experiment.
4. All reagents in the reagent kit should be thoroughly melted and mixed at room temperature before use, and should be centrifuged immediately.
5. The negative and positive controls provided in the reagent kit should be transferred to the specimen preparation area and stored separately before the first use.
6. To prevent fluorescence interference, direct contact with the PCR reaction tube should be avoided, and any labeling on the PCR reaction tube should be avoided.
7. The parameters related to instrument amplification should be set according to the relevant requirements in this manual; Different batch numbers of reagents cannot be mixed.
8. The waste generated during the experimental process should be treated harmlessly before being discarded.