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Streptococcus pneumoniae serotyping nucleic acid multiplex real-time fluorescence PCR detection kit

Product Number: DTK811

Shipping and Storage

1. Transportation: The reagent kit must be transported under frozen conditions.
2. Storage: Store at -20 ± 5℃ 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	25T
Nucleic acid amplification reaction solution Mix-A	450μL
Nucleic acid amplification reaction solution Mix-B	450μL
Nucleic acid amplification reaction solution Mix-C	450μL
Nucleic acid amplification reaction solution Mix-D	450μL
Nucleic acid amplification reaction solution Mix-E	450μL
Nucleic acid amplification reaction solution Mix-F	450μL
Enzyme mixture	300μL
Positive control	200μL
Negative control	1000μL

Note: The ingredients of different batches of reagent kits cannot be mixed!

Description

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

Application

This kit is designed for the qualitative serotyping of Streptococcus pneumoniae from pure cultures or single colonies isolated from clinical specimens, food samples, or preserved bacterial strains. It covers 24 common serotypes, including 1, 2, 3, 4, 5, 6B/6D, 6A/6B, 7F/7A, 8, 9V/9A, 9L/9N, 10A, 11A/11D/11E, 12F/44, 14, 15B/15C, 17F, 18C/18F/18B/18A, 19A, 19F, 20, 22F, 23F, and 33A/33F/37. The experimental results are intended for basic research purposes only and should not be used as a basis for clinical diagnosis.

Streptococcus pneumoniae often resides in the nasopharyngeal cavity of normal individuals and is mostly non pathogenic, with only some having pathogenicity. It can cause diseases such as lobar pneumonia, peritonitis, and pleurisy.

Applicable instruments

The fully automatic fluorescence PCR detector that has undergone multi-channel calibration needs to include FAM, VIC (HEX), CY5, and ROX detection channels, such as ABI7500, 7500FAST, Bio Rad CFX96, Shanghai Hongshi SLAN-96P and other fully automatic fluorescence PCR detectors.

Specimen collection

Pure cultures or single colonies obtained from clinical and food samples, as well as DNA samples extracted from pure cultures that preserve bacterial strains, can all be tested using this kit.

Protocol

1. Perform nucleic acid extraction using commercial DNA extraction reagents. It is recommended that the volume of eluent be no less than 50μL (at least 30μL of nucleic acid is required for screening all 24 serum subtypes). The extracted DNA can be directly used for detection. If the sample is not detected immediately after extraction, it can also be stored at -20℃ or -70℃ for future use. Repeated freezing and thawing should be avoided.

Component	Volume (μL)
qRT-PCR Pre mixed solution (containing enzymes)	16
Primer probe HHV6 / HHV7/ HHV8	4
Total volume (reaction system mixture)	20

2. **Preparation of reaction system**

In order to comprehensively detect the serotypes of Streptococcus pneumoniae covered by the test kit, each sample should undergo PCR amplification in six groups: A, B, C, D, E, and F.

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), a system is prepared with 6× (n+1) reactions, including n+1 reactions in group A, n+1 reactions in group B, n+1 reactions in group C, n+1 reactions in group D, n+1 reactions in group E, and n+1 reactions in group F. The reaction system is prepared as shown in the table below.

Reagent	Quantity of 1 reaction system	Quantity of n+1 reaction system
Nucleic acid amplification reaction solution Mix-A/B/C/D/E/F	18μL	18μL×（n+1）
Enzyme mixture	2μL	2μL×（n+1）

2.2. System packaging

After mixing and centrifuging the reaction solutions of groups A, B, C, D, E, and F, divide them into 20μL tubes and transfer them into PCR tubes suitable for fluorescence PCR machines.

2.3. Sampling

Take 5μL of DNA samples extracted in step 1 and add them to six reaction systems A, B, C, D, E, and F. Tighten the tube cap, mix gently, 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**

Step	Cycles	Temperature	Time	
1	1 cycle	95℃	30sec	
2	40 cycles	95℃	10sec	
		60℃	30sec	Collect fluorescence

Note: Other instruments such as ABI7500 have a fluorescence collection setting of 31 seconds, which has no effect on the results.

Detection settings: "Reporter Dye" is set to FAM, VIC (HEX), CY5, and ROX respectively, and the corresponding channels for each target are detailed in the result interpretation.

Quencher Dyes are all 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. **quality control**

The negative control had no amplification curve, and the positive control had S-shaped amplification curves in the detection channels corresponding to tubes A, B, C, D, E, and F, indicating the validity of the experiment. Otherwise, the experimental results will be deemed invalid.

6. Result analysis and judgment

6.1. 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 in the following table;

6.2. 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, it shall be judged according to the fluorescence channel corresponding to the detection target in the following table. Otherwise, it shall be judged as negative;

6.3. If the sample has no obvious S-shaped amplification curve in the detection channel, but a Ct value is reported, it is still considered negative.

	Thoroughfare	Result interpretation
A pipe	FAM	Streptococcus pneumoniae serotype 1
	VIC/HEX	Streptococcus pneumoniae serotype 3
	CY5	Streptococcus pneumoniae serotype 2
	ROX	Streptococcus pneumoniae serotype 4
B pipe	FAM	Streptococcus pneumoniae serotype 5
	VIC/HEX	Streptococcus pneumoniae serotype 6B/6D
	CY5	Streptococcus pneumoniae serotype 6A/6B
	ROX	Streptococcus pneumoniae serotype 7F/7A
C pipe	FAM	Streptococcus pneumoniae serotype 8
	VIC/HEX	Streptococcus pneumoniae serotype 9V/9A
	CY5	Streptococcus pneumoniae serotype 9L/9N
	ROX	Streptococcus pneumoniae serotype 10A
D pipe	FAM	Streptococcus pneumoniae serotype15B/15C
	VIC/HEX	Streptococcus pneumoniae serotype 14
	CY5	Streptococcus pneumoniae serotype 12F/44
	ROX	Streptococcus pneumoniae serotype 11A/11D/11E
E pipe	FAM	Streptococcus pneumoniae serotype 19A
	VIC/HEX	Streptococcus pneumoniae serotype 17F
	CY5	Streptococcus pneumoniae serotype 18C/18F/18B/18A
	ROX	Streptococcus pneumoniae serotype 19F
F pipe	FAM	Streptococcus pneumoniae serotype 20
	VIC/HEX	Streptococcus pneumoniae serotype 33A/33F/37
	CY5	Streptococcus pneumoniae serotype 22F
	ROX	Streptococcus pneumoniae serotype 23F

Limitations of detection methods

The target sequence detected by this kit is the conserved region of Streptococcus pneumoniae genes, which are highly conserved. But if the target undergoes a genetic mutation at the target sequence, false negative results may occur, that is, missed detection may occur; Meanwhile, the quality of sample collection, processing, transportation, and preservation all have an impact on the test results.

Performance indicators of reagent kit

1. Minimum detection limit: 5×10^2 copies/mL.
2. Linear range: 5×10^2~2×10^{10} copies/mL.
3. Cross reactivity: There is no cross reactivity with other pathogens that may cross with Streptococcus pneumoniae, such as Japanese encephalitis virus, mumps virus, enterovirus, herpes simplex virus, poliovirus, measles virus, respiratory syncytial virus, human cytomegalovirus, EB virus, Listeria monocytogenes, Streptococcus agalactiae, Neisseria meningitidis, Haemophilus influenzae, Micrococcus, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecium, coagulase negative Staphylococcus, Cryptococcus neoformans, and Streptococcus pyogenes.
4. Precision: The coefficient of variation of the reference standard for detecting precision is less than 5%.

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Note

1. Please read the instructions of this reagent kit carefully before the experiment and strictly follow the operating steps.
2. The components 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.