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Poliovirus Types 1/2/3 Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK552

Shipping and Storage

Store at -20℃ away from light, with a shelf life of 12 months. After opening, store in the dark at -20℃ without affecting the shelf life; The number of repeated freeze-thaw cycles should not exceed 5 times.

Component

Component	50T
RT-PCR reaction solution	375μL
Enzyme solution	250μL
Poliovirus type 1, type 2, and type 3 detection solution	375μL
Positive control	50μL
RNAse free water (blank control)	250μL

Note: Different batches of reagents cannot be mixed.

Description

This kit uses real-time fluorescence PCR technology and hydrolysis probe technology to achieve qualitative detection of one or more pathogens at the nucleic acid level in the same reaction tube. Easy to operate, no need to open the lid throughout the process to reduce pollution. The instrument software system automatically draws real-time amplification curves to achieve real-time result judgment.

This kit is equipped with an internal standard, which is ribonuclease P (RNaseP) labeled with CY5 fluorescein. The collection, transportation, and extraction of the test sample are monitored through the internal standard to avoid false negatives in the test results.

Application

This kit is used for qualitative detection of poliovirus types 1, 2, and 3 nucleic acids in throat swabs and other samples. The test results are only used for clinical auxiliary diagnosis and cannot be used alone as a basis for confirming or excluding cases.

Poliovirus belongs to the enterovirus genus of the Picornaviridae family, and has three serotypes: type 1, type 2, and type 3. It is mainly transmitted through the digestive tract and can also be transmitted through nasal droplets. Poliovirus often invades the central nervous system, mainly causing poliomyelitis, characterized by damage to the spinal cord anterior horn motor nerve cells and limb flaccid paralysis. As it is more common in children, it is also known as poliomyelitis. The clinical symptoms vary in severity, with most cases being mild or asymptomatic, and a few patients may experience flaccid paralysis. The laboratory testing methods mainly include virus isolation, immunological testing, nucleic acid testing, etc.

Applicable instruments

ABI7500, QuantStudio™ 5, QuantStudio™ 7, RocheLightCycler® 480, Bio-Rad CFX96™, Shanghai Hongshi SLAN-96P/S fluorescence quantitative PCR instrument.

Specimen collection

1. Sample type: throat swab, etc.
2. Collection method: Pharyngeal swab samples: The collection of pharyngeal swab samples shall be carried out in accordance with the pharyngeal swab collection method specified in the Clinical Nursing Practice Guidelines.
3. Storage method: After sample collection, it should be tested in a timely manner. The test should be completed within 3 days of

storage at 2℃ -8℃, 4 months at -20±5℃, and 12 months at -70℃. The sample should avoid repeated freeze-thaw cycles, with no more than 5 freeze-thaw cycles, and should not be transported in ice packs at low temperatures for more than 3 days.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

Take out the reagent kit, melt and shake at room temperature, centrifuge at low speed for 10 seconds, and calculate the number of reactions required (n=number of samples+2 tubes of control). The preparation of the reaction system per person is as follows:

Composition of reaction solution	Dosage (μL)/per reaction
RT-PCR reaction solution	7.5μL
Enzyme mixture	5μL
Poliovirus type 1, type 2, and type 3 testing solution	7.5μL
Total volume	20μL

Calculate the usage amount of each reagent mentioned above, add it to an appropriate volume of centrifuge tube, mix thoroughly, and then divide it into n PCR reaction tubes in a quantity of 20μL.

2. Sample processing (sample processing area)

2.1. Nucleic acid extraction

Take the test sample and extract nucleic acid according to the instructions of the nucleic acid extraction kit. We recommend using our company's virus nucleic acid extraction kit for nucleic acid extraction.

2.2. Sampling

Add 5μL of processed test sample nucleic acid, positive control, and blank control to the prepared PCR reaction tube, with a final volume of 25μL/tube. Cover the tube tightly and centrifuge at low speed instantly.

3. Reagent preparation (reagent preparation area)

3.1. Place the reaction tube into a fluorescent PCR amplifier for amplification detection.

3.2. Taking ABI7500 operation instructions as an example.

3.3. Loop parameter setting:

step		Temperature	Time	Cycles
1	Reverse transcription	50℃	10min	1 cycle
2	Pre denaturation	95℃	5min	1 cycle
3	Denaturation	95℃	10sec	45 cycles
	Annealing, extension, and fluorescence detection	58℃	30sec	
Fluorescence detection at 58℃ in step 3, detection channel: FAM、VIC、ROX、CY5				

Note: The ABI7500 real-time fluorescence PCR instrument does not select ROX calibration, and the quenching group is selected as None.

3.4. Result analysis

ABI 7500: After the reaction is completed, the results will be automatically saved. Based on the analyzed image, adjust the Start value, End value, and Threshold value of the BaseLine (which can be adjusted according to the actual situation. The Start value can be set between 3-15, and the End value can be set between 5-20. Adjust the amplification curve of the blank control to be flat or lower than the threshold line). Click on Analysis to automatically obtain the analysis results, and view the results on the Report interface.

4. Positive judgment value

4.1. Positive: Ct ≤ 37 and the curve is S-shaped.

4.2. Negative: Ct>40 or not detected.

5. Interpretation of Inspection Results

5.1. Quality control

Channel	Ct value			
Control group	FAM channel	VIC channel	ROX channel	CY5 channel
Blank control	Undet	Undet	Undet	Undet
Positive control	Ct≤30	Ct≤30	Ct≤30	Ct≤30

5.2. Result interpretation

Under the condition that the instrument is normal and both positive and negative controls are normal, analyze the results according to the table below.

Channel	Pathogens/Genes	Interpretation of Results
FAM	Poliovirus type 1	1. Positive: The test result Ct ≤ 37, with an S-shaped curve and a significant exponential growth period, is judged as positive for the corresponding pathogen/gene; 2. Negative: If the Ct test result is greater than 40 or not detected, this result is judged as negative for the corresponding pathogen/gene. 3. Suspicious: The test result of the sample to be tested is 37 ＜ Ct ≤ 40. At this point, the sample should be retested. If the Ct value of the re read experiment is still within the range of 37-40 and the curve shows a standard S-shape with a significant exponential growth period, it is considered positive for the corresponding pathogen/gene. Otherwise, it is considered negative for the corresponding pathogen/gene.
VIC	Poliovirus type 2	
ROX	Poliovirus type 3	
CY5	Internal standard	The detection result of the CY5 channel of the sample internal standard should have Ct ≤ 37, otherwise sampling and retesting are required. When the sample result is interpreted as positive, if the internal standard Ct is greater than 40 or not detected, the result is still reliable.

Limitations of detection methods

1. This kit is used for qualitative detection of nucleic acids in samples. The clinical diagnosis and treatment of patients should be comprehensively considered based on their symptoms/signs, medical history, other laboratory tests, and treatment response.
2. Analysis of the possibility of false positive results: Cross contamination of samples during transportation and processing; The experimental environment is contaminated with aerosols such as PCR products; The contamination of consumables, equipment, etc. used during the experimental process may lead to false positive results.
3. Analysis of the possibility of false negative results

   Incorrect sample collection, transportation, and processing; The pathogen content in the sample is too low; Sequence changes caused by variations or other reasons in the target sequence of the pathogen to be tested; Other unverified interferences or PCR inhibitors may lead to false negative results.

Product performance indicators

1. Minimum detection limit: 500 copies/mL;
2. Linear detection range: 2×10^3~1×10^8 copies/mL;
3. Specificity: There is no cross reactivity between poliovirus types 1, 2, and 3, and there is no cross reactivity in the total nucleic acid of other common pathogens (human coronavirus, influenza A virus, influenza B virus, parainfluenza virus, respiratory syncytial virus, human adenovirus, Haemophilus influenzae, Streptococcus pneumoniae, Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae, Neisseria meningitidis, Japanese encephalitis virus, Escherichia coli, mumps virus) and human white blood cells that have the same infection site or similar infection symptoms.
4. Precision: The coefficient of variation of the reference standard for detecting precision is less than 5%.

Note

1. This reagent kit is for scientific research use only. Operators should receive professional training and have certain experience.
2. To ensure the accuracy and reliability of the experimental results, please use a calibrated pipette and select qualified disposable PCR reaction tubes, centrifuge tubes, gun heads, etc. for sample processing and liquid preparation operations. All tools should be free of DNA enzymes and RNA enzymes.
3. Please strictly partition the experiment; All items and work clothes in each district are for exclusive use and must not be used interchangeably to avoid contamination. Please clean the workbench immediately after the experiment.
4. This product should be fully melted at room temperature, mixed well, and centrifuged at low speed immediately before use.
5. Sample processing should be carried out in a biosafety cabinet to protect the safety of operators and prevent environmental pollution.

6. Blank control and positive control should be set up for each experiment. Do not mix reagents of different batches. Use the reagent kit within its expiration date.
7. The test sample should be kept as fresh as possible, and the extraction process should strictly prevent RNA enzyme contamination and RNA degradation caused by improper operation.
8. RNA samples stored at -70℃ should be thoroughly melted, mixed, and centrifuged at low speed at room temperature before use.
9. The reaction tubes containing reaction solution should be capped or packed in a compact bag before being transferred to the sample processing area.
10. When adding the sample, it should be completely added to the reaction solution, and there should be no sample adhering to the tube wall. After adding the sample, the tube cover should be tightly closed as soon as possible.
11. When packaging the reaction solution, try to avoid generating bubbles as much as possible. Before starting the machine, pay attention to checking whether each reaction tube is tightly covered to avoid leaking and contaminating the instrument.
12. After amplification, remove the reaction tube, seal it in a special plastic bag, and discard it at the designated location.
13. Please directly pour the gun head used in the experiment into the waste tank containing 10% sodium hypochlorite and discard it together with other waste items.
14. Workbenches and various experimental items are often disinfected with 10% sodium hypochlorite, 75% alcohol, and ultraviolet lamps.
15. The real-time fluorescence PCR instrument requires frequent calibration and cleaning of the sample plate holes.
16. The test samples involved in this kit should be considered as infectious substances, and the operation and handling must comply with the relevant requirements of the Ministry of Health's "General Guidelines for Biosafety of Microbial Biomedical Laboratories" and "Regulations on the Management of Medical Waste".