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Eight Types of Viral Diarrhea Pathogens Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK543

Shipping and Storage

1. Store at -20℃ away from light, with a shelf life of 12 months.
2. Low temperature transportation cannot exceed 4 days; After opening, store in the dark at -20℃ without affecting the expiration date. Avoid repeated freezing and thawing, freezing and thawing 6 times will not affect the detection effect.

Component

Component	Specification and quantity		Main components
	25T	50T	
qRT-PCR reaction solution	300μL×3	600μL×3	Tris, KCl, $MgCl_2$, dNTPs, etc
qRT-PCR enzyme mixture	100μL×3	200μL×3	Reverse transcriptase, RNAse inhibitor, Taq enzyme, etc
① Tube Primer Probe NVGI/NVGII	100μL	200μL	Primer probe
② Tube Primer Probe RVA/RVB/RVC	100μL	200μL	Primer probe
③ Tube Primer Probe EAdV/AstV/SaV	100μL	200μL	Primer probe
Positive Control (Eight Diarrhea Pathogens)	200μL	200μL	Plasmids containing target detection gene fragments
Negative control	200μL	200μL	Water treated with diethyl carbonate

Note:

① **Components from different batch numbers cannot be interchanged.**

② **Self provided reagents: Nucleic acid extraction kit, we recommend using our company's nucleic acid extraction and purification kit for extraction.**

③ **The detection targets of each channel of the three tube primer probe are as follows:**

Name	FAM channel	VIC channel	CY5 channel
① Tube Primer Probe	Norovirus type I	Norovirus type II	
② Tube Primer Probe	Rotavirus Group A	Rotavirus Group B	Rotavirus Group C
③ Tube Primer Probe	Enteral adenovirus	Human astrovirus	Zharu virus

Description

This kit uses real-time fluorescence PCR technology to target the relatively conserved regions of eight common diarrhea viruses: Norovirus type I, Norovirus type II, Rotavirus group A, Rotavirus group B, Rotavirus group C, enteroadenovirus, human astrovirus, and Zharu virus. Specific primers and probes are designed for rapid detection of RNA/DNA of the eight viruses through three tubes. After nucleic acid extraction, the sample is added to the detection reagent provided in the kit to prepare a PCR reaction tube. The fluorescence quantitative PCR instrument is used for one-step RT-PCR amplification, and the fluorescence signal is detected. The instrument software system automatically draws a real-time amplification curve.

Application

This product is used for qualitative detection of nucleic acids of Norovirus Type I/NVGI, Norovirus Type II/NVGII, Rotavirus Group A/RVA, Rotavirus Group B/RVB, Rotavirus Group C/RVC, Enteroadenovirus/EAdV, Human Astrovirus/AstV, and

Jarovirus/SaV. The test results are used for auxiliary diagnosis of diarrhea pathogens in clinical practice and cannot be used alone as a basis for confirming or excluding cases.

Diarrhea infection is a common clinical condition that can be caused by various viruses. Common diarrheal viruses include: diarrheal syncytial virus, diarrheal adenovirus, human papillomavirus, parainfluenza virus, influenza A virus, influenza B virus, etc. Diarrhea virus infection can cause various diseases, such as colds, influenza, pharyngitis, laryngitis, bronchitis, pneumonia, etc. The main clinical symptoms of human infection include fever, runny nose, sore throat, cough, headache, and diarrhea. Due to the similarity in infection symptoms and epidemic characteristics caused by these viruses, it is highly unreliable to distinguish pathogens solely based on clinical symptoms. Laboratory diagnostic methods include virus isolation, antigen testing, and nucleic acid testing.

Applicable instruments

ABI 7500 Fluorescence Quantitative PCR Instrument.

Specimen collection

1. The applicable sample types are feces, vomit, and other samples.
2. After sample collection, it should be tested in a timely manner. The test should be completed within 3 days of storage at 2℃~8℃. If it exceeds 3 days, it should be stored at -40℃ or below for more than 6 months without affecting the test results. Avoid repeated freezing and thawing, freezing and thawing 6 times will not affect the test results.

Protocol

1. Reagent Preparation (Reagent Preparation Area)

Take out qRT-PCR reaction solution, qRT-PCR enzyme mixture, ① tube primer probe (② tube primer probe, ③ tube primer probe) from the reagent kit, melt at room temperature, shake thoroughly and mix well, then centrifuge instantly. Calculate the number of reagents used N (N=number of samples+1 tube of positive control+1 tube of negative control), configure the reaction system according to the table below, add it to an appropriate volume of centrifuge tube, shake thoroughly and mix, centrifuge instantly, divide it into 20μL PCR reaction tubes, and transfer it to the sample processing area (tubes ①, ②, and ③) to prepare the above N reagents.

Component	Add volume (μL) per person
qRT-PCR reaction solution	12μL
qRT-PCR enzyme mixture	4μL
① Tube Primer Probe (② Tube, ③ Tube)	4μL
Total Volume	20μL

2. Sample processing (sample processing area)

2.1. Nucleic acid extraction: The test sample should be operated according to the instructions of the nucleic acid extraction kit.

2.2. Sample addition: Add 5μL of the test sample nucleic acid, positive control, and negative control to the prepared PCR reaction tube, with a final volume of 25μL/tube. Cover the tube tightly and centrifuge immediately.

3. PCR amplification detection (nucleic acid amplification zone)

3.1. Place the PCR tube in a fluorescent PCR amplifier for amplification and detection.

3.2. Loop parameter setting

	Step	Temperature	Time	Cycles
1	Reverse transcription	50℃	10min	1 cycle
2	Pre denaturation	95℃	5min	1 cycle
3	Sex change	95℃	10sec	40 cycle
	Annealing/extension/fluorescence detection	55℃	40sec	

Note:

① **Set fluorescence detection at 55℃ in step 3.**

② **ABI7500 fluorescence PCR instrument does not select ROX calibration, and the quenching group is selected as None.**

4. Result analysis parameter setting

Threshold setting: Adjust the Start value, End value, and Threshold Value of the Baseline based on the analyzed image (Start value is recommended to be set at 3-15, End value is recommended to be set at 5-20, and adjust the amplification curve of the negative control to be flat or below the threshold line). Click on Analysis to automatically obtain the analysis results, and view the results on the Report interface.

Positive judgment value

According to the results of clinical sample testing, the critical Ct values of 12 viruses in this kit were determined to be 35 using the ROC curve method.

Interpretation of Inspection Results

1. Quality control standards

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

	Passageway	Negative control	Positive control
① Tube	FAM Channel (NVGI)	No Ct value	Ct≤30
	VIC Channel (NVGII)	No Ct value	Ct≤30
② Tube	FAM Channel (RVA)	No Ct value	Ct≤30
	VIC Channel (RVB)	No Ct value	Ct≤30
	CY5 Channel (RVC)	No Ct value	Ct≤30
③ Tube	FAM Channel (EAdV)	No Ct value	Ct≤30
	VIC Channel (AstV)	No Ct value	Ct≤30
	CY5 Channel (SaV)	No Ct value	Ct≤30

2. Result interpretation

2.1. Analyze and interpret the test results of the test sample when the instrument is normal and the positive control, negative control, and internal standard control test results meet the quality control standards.

2.2. Positive interpretation: If the amplification curve of the test sample shows a typical S-shaped curve and meets the following conditions, it is judged as positive.

	Passageway	Ct value	Result interpretation
① Tube	FAM	Ct≤35	Norovirus type I positive
	VIC	Ct≤35	Norovirus type II positive
② Tube	FAM	Ct≤35	Rotavirus Group A positive
	VIC	Ct≤35	Rotavirus Group B positive
	CY5	Ct≤35	Rotavirus Group C positive
③ Tube	FAM	Ct≤35	Gut adenovirus positive
	VIC	Ct≤35	Positive for human astrovirus
	CY5	Ct≤35	Zharu virus positive

3. Negative interpretation: If the FAM channel, VIC channel, or CY5 channel is greater than 38 or there is no obvious logarithmic growth period in the amplification curve, it is judged as negative.

4. Retest: If there is a logarithmic growth phase in the amplification curves of FAM channel, VIC channel, or CY5 channel, and the Ct value is between 35 and 38, retesting is required; If the retest results are consistent, the judgment result is positive.

Limitations of testing methods

1. The test results of this kit are for clinical reference only. 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 false negative results:

2.1. Unreasonable sample collection, transportation, and processing, as well as low virus titers in samples, can all lead to false negative results.

2.2. Variations in the target sequence of the diarrhea virus or sequence changes caused by other reasons may result in false

negative results.

2.3. For sudden outbreaks of diarrhea virus, the optimal sample type for detection and the optimal sampling time after infection may not have been confirmed. Therefore, collecting samples from multiple locations in the same patient can reduce the possibility of false negative results.

3. The nucleic acid sequence to be tested may appear in the body for a long time and is not related to viral activity. A positive nucleic acid test does not necessarily indicate current infection with the corresponding virus or its clinical symptoms as a pathogenic factor.

Product performance indicators

1. Minimum detection limit: 1 × 103 copies/mL.
2. Linear detection range: 2 × 103~1 × 108 copies/mL.
3. Specificity: No cross reactivity to other pathogenic bacteria.

Note

1. Before testing, it is necessary to carefully read this manual and strictly follow the requirements for operation.
2. This kit is an in vitro detection reagent, and the operation requires a certain level of professionalism. Operators should receive professional training.
3. The pathogen nucleic acid detected by this kit is RNA/DNA, and all consumables used during the operation should not contain DNA enzyme and RNA enzyme.
4. Laboratory operations shall be carried out in accordance with the "Management Measures for Clinical Gene Amplification Testing Laboratories in Medical Institutions". Experimental operations must be strictly partitioned, and the instruments, equipment, consumables, and work clothes used in each area must be dedicated and not cross used to avoid contamination.
5. The components of different batch numbers in the reagent kit cannot be interchanged. Before use, each component should be melted at room temperature, thoroughly shaken and mixed, and immediately centrifuged before use.
6. When packaging the reaction solution, avoid generating bubbles, and check whether each reaction tube is tightly covered before starting the machine.
7. After the experiment is completed, the table should be cleaned in a timely manner and disinfected with measures such as 10% sodium hypochlorite, 75% alcohol, and ultraviolet light.
8. The test samples involved in the reagent kit should be considered infectious, and all laboratory operations should comply with the “General Biosafety Guidelines for Pathogenic Microbial Laboratories”; The disposal of medical waste should be managed in accordance with the “Regulations on the Management of Medical Waste”.
9. This kit is only used for scientific research purposes and is not intended for clinical diagnosis.