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Enterovirus Nucleic Acid Detection Kit

(Fluorescent Probe-Based Real-Time PCR Assay)

Instructions for Use

English VER 2.0

REF E016T024B0C1 24 Tests/Box

PRODUCT NAME

Enterovirus Nucleic Acid Detection Kit (Fluorescent Probe-based Real-time PCR Assay)

INTENDED USE

This kit is used for qualitative detection of enterovirus nucleic acid RNA in human feces, cerebrospinal fluid and oropharyngeal swab samples. For research use only.

SUMMARY AND EXPLANATION

Enterovirus belongs to the Picomaviridas family, including poliovirus, Coxsackie virus, Echo virus and new enteroviruses. Human enterovirus is a single-stranded positive-stranded RNA virus, the virus particles are spherical, icosahedral three-dimensional symmetry, without envelope. The genome is about 7000 bp in length, including non-coding regions at the 5' and 3' ends and a large open reading frame (ORF) in the middle. Humans are the only host of human enterovirus, and the infection can lead to a variety of diseases, including paralytic disease, aseptic meningitis, myocardial injury, hand, foot and mouth disease, conjunctivitis, and rash. Patients and hidden infections are the source of infection, and hidden infections are difficult to identify. This kit detects enterovirus nucleic acid RNA by detecting the 5'-untranslated region sequence, which is used for the auxiliary diagnosis of human enterovirus infection. Clinical or laboratory diagnosis uses virus cell culture separation and neutralization test as the main method to detect universal enterovirus.

TEST PRINCIPLE

This kit uses polymerase chain reaction (PCR) combined with Taqrnan fluorescent probe technology to identify and detect universal enteroviruses in human feces, cerebrospinal fluid and oropharyngeal swab samples. The enteroviruses that can be detected by this kit include: Coxsackie virus A group CA2, CA4-7, CA9, CA10, CA12 and CA16, Coxsackie virus B group CB1-5, Echo virus ECHO6, ECHO9-11, ECHO13-21, ECHO24-25, ECHO30, ECHO33, poliovirus polivirus1-3 and human enterovirus 90 and 71.

REAGENTS PROVIDED

Seq.	Labels	Main Contents	*No.* (24 tests)
1	Weak Positive Control (EV)	Containing enterovirus gene fragments	1 tube(500μL/tube).
2	Strong Positive Control (EV)	Containing enterovirus gene fragments	1 tube(500μL/tube).
3	Internal Control (EV)	Containing internal control gene fragments	1 tube (130μL /tube).
4	Buffer (EV)	Containing magnesium ion, deoxynucleotide solution	1 tube (305μL /tube).
5	DNA Polymerase (EV)	Contains DNA polymerase solution	1 tube(13μL/tube).
6	Reverse Transcriptase (EV)	Containing reverse transcriptase solution	1 tube(13μL/tube).
7	Primers/Probes (EV)	Contains specific primers and probe solutions	1 tube(40μL/tube).

OTHER MATERIALS REQUESTED BUT NOT PROVIDED

The following list includes the materials that are required for use but not included in this Kit:

- Nucleic acid extraction kit.
- Nuclease-free consumables: Filter tips, 1.5mL tubes, PCR-well strips or 96-well plate.
- Experimental equipment: Centrifuge for 1.5mL tubes and PCR-well strips or 96-well plate (if available), Vortex. Real Time PCR instrument (thermocycler).
- Others: Micropipettes (0.5-20μL, 10-100μL, 20-200μL,100-1000μL), Powder-free disposable gloves, Microplate sealing film.

STORAGE CONDITIONS AND SHELF LIFE

The shelf life of this kit is 6 months when stored in freezer at - (20±5) ℃. It is suggested to transport the kits in a sealed foam boxes with dry ice and/or ice packs. The kit needs to be stored away from light. Never leave the kit for more than3 days at 37℃. Never repeat freeze-thaw more than 3 times for this kit (The effects of repeated freeze-thaw more than 3 times for this kit haven't been verified). Once the bottle is opened, use within 6 days.

APPLICABLE EQUIPMENT

Applicable to ABI 7500 Real-Time PCR thermocycles, for other Real-Time PCR thermocyclers, please consult the manufacturer/your distributor before use.

ACCEPTABLE SPECIMENS

The specimens to be collected are the feces, cerebrospinal fluid or oropharyngeal swab specimens.

SPECIMENS COLLECTION AND STORAGE

Feces: The samples are collected within 3 days of the patient's onset, and the collection amount is 5-8g/piece;

Oropharyngeal swab: The samples are collected within 3 days of the patient's onset. With a special swab wipe moderately the back wall of the pharynx and the two of the tonsils. After collection, quickly insert the swab into a sample tube containing 3 mL of preservation solution and close the tube cap.

Cerebrospinal fluid: Before the cerebrospinal fluid collection, the patient should be fasted, and the doctor should use aseptic procedures to puncture the patient's 3rd and 4th lumbar intervertebral spaces or slightly lower, and for children, puncture the 4th and 5th lumbar intervertebral spaces. It must

be put into a sterile container with a cap (lid), stored at 2-8°C, and sent for inspection immediately.

Pay attention to aseptic operation during sample collection. During the transportation and storage of clinical specimens, repeated freezing and thawing should be avoided. If the condition of -(20±5)℃ cannot be ensured, it should be transported at below 8℃. The shelf life at -(20±5)℃ is 4 months. During transportation and storage, the necessary information should be attached, such as the specimen number, the date of onset, and the date of specimen collection.

TEST METHODOLOGY

1. Nucleic Acid Extraction (Pre-PCR)

Extract the nucleic acids from the clinical samples, positive controls and negative control according to the instructions of the nucleic acid extraction kit:

1.1. Clinical sample: the clinical sample to be tested;
1.2. Positive Control: Strong positive control (EV) and weak positive control (EV) are included in this kit.
1.3. Negative Control: normal saline or sterile water supplied by the user.

Note: Take 200μL of each of the above three quality control materials and process them in parallel with the samples. Before nucleic acid extraction, add the internal control to the lysis solution according to the amount of 5μL/test.

*We have validated the following kits for nucleic acid extraction from feces, cerebrospinal fluid or oropharyngeal swab specimen:

- Nucleic Acid Extraction Kit, using the 32M, 96M or FAST96 instruments or extract manually (Mole Bioscience). recommended*.
- Nucleic Acid Extraction Kit (Spin Column) (Mole Bioscience). recommended*

If you use nucleic acid extraction kits from other suppliers, please verify first.

2. Amplification Processes (PCR)

2.1 Preparation of Amplification Reagent (PCR Room I)

To prepare the PCR reaction mixtures, take the buffer (EV), DNA polymerase (EV), reverse transcriptase (EV) and Primers/Probes (EV) from the kit. Thaw on ice or at 2-8℃. Shake well and centrifuge all reagent tubes at low speed shortly. Prepare the Amplification PCR Mixture according to the following ratios:

Reagent	Buffer (EV)	DNA Polymerase (EV)	Reverse Transcriptase (EV)	Primers/Probes (EV)
Volume	12.5μL	0.5μL	0.5μL	1.5μL

Calculate the amount of each reagent. Add the reagents into an appropriate volume centrifugal tube, mix well and centrifuge shortly. Total number of PCR mixtures = number of samples + 2 positive control +1 negative control.

Add 15.0μL of the PCR mixture into each of the PCR tube, and then transfer the plates/tubes to the sample processing area.

2.2 Add the Templates (PCR Room II)

Add 10.0μL of nucleic acid extracted from each sample (prepared in the first step) into each PCR well/tube which were added with PCR mixture. Vortex the sealed plate or tubes to mix well and then centrifuge at 2000-3000rpm for 1min.

2.3 Amplification (Detection Area)

Put the reaction tube into the fluorescence PCR detector, and set the cycle parameters as follows:

Steps	Cycles	Temperature (℃)	Time (min: sec)
1	1	45	15:00
2	1	95	02:00
3	40	94	00:10
		60*	00:40

Fluorescent dye signals assigning: FAM (enterovirus) and HEX/VIC (internal control). *The signal data is collected at 60°C. When using the ABI7500, select the 'Quencher' and 'Passive reference' columns as "none". Set the reaction volume per tube/well to 25 μL.

CUT-OFF VALUE

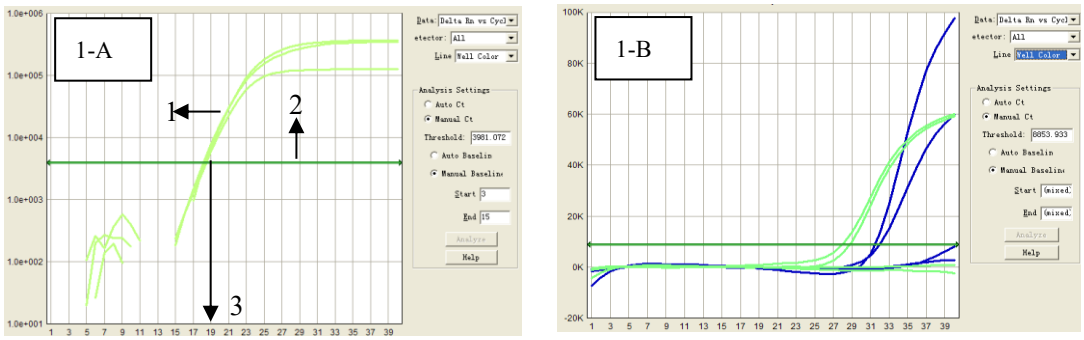
The cut-off value of FAM is 36.70, it is determined by ROC curve.

EXPLANATION OF THE TEST RESULTS

After the reaction is over, the instrument automatically saves the results, and after analyzing the image, adjust the Start value, End value and Threshold value of Baseline (self-adjustable, start value can be between 3-15, End value can be between 5-20.).

For negative control, the Ct value in FAM should be negative. For strong positive control, the Ct value in FAM should be between 16-23. For weak positive control, the Ct value in FAM should be between 28-35. The Ct value in HEX/VIC (internal control) should be ≤35. The above conditions must be met at the same time in the same test, otherwise the PCR reaction is considered invalid and the test needs to be repeated. Details as follows:

1. Negative sample: If the result shows negative or the FAM Ct > 38.19 or no typical S-type amplification curve for the tested sample, the tested sample is determined as negative.
2. Positive Sample: If the result shows negative or the FAM (CA16) Ct ≤ 35.20, with a typical S-type amplification curve, the tested sample is determined as a positive sample.
3. Suspicious positive samples: If the tested sample shows that the value of FAM 35.20 ≤ Ct ≤ 38.19, and with a typical S-type amplification curve, the tested sample is considered as suspicious positive. For suspicious samples, a secondary test should be performed. If the secondary test shows that Ct value of FAM ≤ 38.19, the tested sample is determined as positive, otherwise, these sample is judged as negative. When the secondary test is carried out, the further concentrating and purifying of the sample may increase the detection sensitivity. Because this kit does not have the reagents for concentrating, the extend of the positive impact on the sensitivity has not been verified.
4. If there is a typical S-amplification curve but the concentration is too high and the internal control is not amplified, it can be directly reported as a positive sample, or the sample can be re-measured by 10-fold dilution.
5. Amplification diagram and analysis example of kit detection.



Sig 1：Amplification curve
1-A: Index amplification(log view)，‘1’: amplification curve，‘2’: threshold，‘3’: Ct values.
1-B: Index amplification (linear view)

LIMITATIONS OF THE TEST METHOD

In this kit, a negative result indicates the pathogen concentration in the sample is lower than the detection limitation of the kit.

The following conditions can also cause a false positive or false negative test result:

1. The results can be affected by collecting, disposing, transporting and storage of samples, and any errors of these processes will result in false negative.
2. The mutation of sequence related to primers or probes used in this kit may cause false negative results.
3. Cross-contamination during sample processing may lead to false positive, and the FAM channel detection results of the negative control showed an amplification curve.

PRODUCT PERFORMANCE

1. According to the comparison results of product clinical trials, the positive coincidence rate of this kit for throat swab samples is 95.69%; the positive coincidence rate for stool samples is 94.17%; the negative coincidence rate for throat swab samples is 96.63%; The negative coincidence rate for stool samples was 94.69%. When the sample concentration is too low (Ct>35 or so), 10 repeated tests will not be 100% positive.
2. Other viruses with the same site of infection or similar symptoms of infection (such as varicella zoster virus, influenza virus A, influenza virus B, Adenovirus, herpes simplex virus, Rhinovirus, Mycoplasma pneumoniae, Chlamydia pneumoniae, HBV, HCV, *Streptococcus*, *Staphylococcus aureus* and *Pneumococcus*) have no cross-reactivity.
3. In the tested sample, when the concentrations of common interfering substances such as blood, pus and the medication drugs for hand-foot-mouth disease treatment such as methylprednisolone, acyclovir and ribavirin, is 10%, 5%, 0.1g•L^{-1}, 0.1g•L^{-1} and 0.1g•L^{-1}, respectively, the performance of the kit will not be affected.

PRECAUTIONS

1. The test is manually operated. Experimental personnel who perform this test should have received professional training in gene amplification or molecular biology diagnostics and be qualified for relevant experimental operations. There should be reasonable biosecurity precautions and protective procedures in the laboratories. The test should only be performed in laboratories that follow safety practices according to the applicable Biosafety Regulations in Microbiological and Biomedical Laboratories.
2. The whole detection process should be carried out in three areas: the first area is for reagent preparation. The second area is for specimen processing and reaction system preparation. The third area is for amplification, fluorescence detection and results analysis. Instruments, equipment and lab coats should be used independently in each area to prevent contamination.
3. In the testing process, should always take care to avoid RNase contamination, wear disposable gloves without fluorescent substances (Frequent replacement is recommended), use the disposable thin-walled 200μL PCR tube (or 96-well PCR plate with optical film) and pipette tips with filter. Never touch the reaction tube directly with bare hands.
4. The handling of Clinical Specimens should be performed in the biosafety cabinet to ensure safety of laboratory staff and prevent environmental pollution. Harmful and/or toxic specimens and reagents in the experiment should be properly placed and stored, and in charge by an assigned person. Waste should be disposed of properly in special containers. Lab bench, equipment such as operator's stations, pipettes, centrifuges, and PCR thermocyclers etc., should be regularly wiped and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol. Laboratory room, ultra-clean bench should be treated with ultraviolet lamp regularly and after each experiment.
5. Prior to the experiment, reagents should be fully thawed, mixed well, and centrifuged for a few seconds to bring down all the liquid to the bottom of the centrifuge tubes. When preparing the reaction solution, attention should be paid to mixing all liquids on the vortex mixer, not blowing with the pipette to avoid bubbles, and centrifuging the reaction mixture solution for a few seconds. Use the kit before the expiration date and do not combinate the reagents with different batch numbers.

REFERENCIAS

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Manufacturing date and expiration date: view on label

INDEX OF SYMBOLS

	Consult Instructions for Use		Manufacture Date	LOT	Lot Number
-25℃ -15℃	Temperature limit -25 to -15°C	Σ	Contain <n> tests		Manufacturer
REF	Catalogue #		Use-by date		

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FOR RESEARCH USE ONLY

Number: 301100050900
Effective Date: 2022-08-01

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变更历史

版本号	生效日期	本次变更原因，依据及详细变更内容
0.0	2021-11-05	初始版本
1.0	2022-01-12	修改抽提试剂，修改语句错误
2.0	2022-08-01	增加脑脊液的样本类型，修改说明书排版，修改提取试剂名称，删除性能处企参相关信息