# Enterovirus/Coxsackievirus A16/Enterovirus 71 RNA Diagnostic Kit (PCR-Fluorescence Probing)

**Product Identification**

Product Name: Enterovirus/Coxsackievirus A16/Enterovirus 71 RNA Diagnostic Kit (PCR-Fluorescence Probing)
Reference Number: S3053E,
Package Specification: 24 tests/kit, Pre-packaged 12 tests/kit

**Intended Use**

The Enterovirus/Coxsackievirus A16/Enterovirus 71 RNA Diagnostic Kit (PCR-Fluorescence Probing) is intended to detect Enterovirus, Coxsackievirus A16 and Enterovirus 71 in throat swab by applying real-time quantitative PCR technique. The detection results can be used to distinguish Coxsackievirus A16 and Enterovirus 71.

**For in vitro diagnostic use only. For professional use only.**

**Test principle summary and explanation**

**Summary**

Hand-foot-and-mouth disease is a common childhood disease caused by enteroviruses. Most of the patients will have a fever and get vesicle rash in the mouth or on the hand and foot. A few patients will get cephalomeningitis, cerebritis, neurogenic pulmonary edema and myocarditis, etc. Some patients may get sequelae of this disease or even death. The viruses that can cause hand-foot-and-mouth disease include Coxsackievirus, new-type enterovirus and ECHO virus, of which the most common types are Coxsackievirus A16 and Enterovirus 71. The laboratory diagnosis methods mainly include virus isolation, nucleic acid detection,etc.

**Test Principle**

By applying real-time fluorescence quantitative PCR technique, this diagnostic kit uses specific primers and fluorescence probes which are designed to target 5'-end untranslated region of Enterovirus, and capsid protein gene of Coxsackievirus A16 and Enterovirus 71, accompanied with other ingredients of EV/CA16/EV71-PCR mix, to achieve quick detection of Enetrovirus, Coxsackievirus A16 and Enterovirus 71 in one tube through fluorescent signal changes.

The PCR detection system uses internal control to monitor the presence of PCR inhibitors in order to avoid a false negative result.

**Materials provided**

This kit is an amplification reaction reagent and contains the following components:

| No. | Reagent Name | Specification & Qty. | | Main Ingredients |
|---|---|---|---|---|
| | | 24 T | Pre-packaged 12T | |
| 1 | EV/CA16/EV71-Internal Control | 50 μL/tube × 1 tube | 1 μL/tube × 12 tubes | Lentivirus diluent |
| 2 | EV/CA16/EV71-PCR Mix | 912 μL/tube × 1 tube | 38 μL/tube × 12 tubes | Primer (8-20 pmol), probe (4-12 pmol), dNTPs (2.1 mmol/L), 5 x PCR buffer (26.3%), DEPC water (60.0%), MgCl2 (1mol/L) |
| 3 | EV/CA16/EV71-Enzyme Mix | 48 μL/tube × 1 tube | 2 μL/tube × 12 tubes | mMLV enzyme (50 U/μL), H-Taq enzyme (2.5 U/μL) |
| 4 | EV/CA16/EV71-Negative Control | 500 μL/tube × 1 tube | 500 μL/tube × 1 tube | Normal saline |
| 5 | EV/CA16/EV71-Positive Control | 500 μL/tube × 1 tube | 500 μL/tube × 1 tube | Lentivirus diluent |

**Materials required but not provided**

1. Materials required but not provided: 1.5 mL DNase-free and RNase-free centrifuge tubes, 0.2 mL PCR reaction tubes, pipette tips (10 μL, 200 μL and 1000 μL tips with filters are preferred), desktop centrifuge, desktop vortex mixer various models of pipettes.
2. Reagent required but not provided Normal saline, Nucleic Acid Extraction-Purification Kit (Reference Number: S50016E Series) or purification kit (Magnetic beads method) (Reference Number: S10011E)

**Warnings and precautions**

**Warnings**

1. Do not mix or exchange components from different kits.
2. All biological materials in the kit have been inactivated.

**Precautions**

1. For *in vitro* diagnostic use only. Please read the product manual carefully before operation.
2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control is performed for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory, laboratories personnel must receive professional training, test processes must be performed in separated regions, all consumables should be for single use only after sterilization, special instruments and devices should be used for every process, all lab devices required in different processes and regions should not be cross-used.
4. All samples for detection should be handled as potentially infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of samples and waste must meet relevant requirements outlined in local, state and national regulations.
5. Note: Improper operation during the storage, transportation and use of the reagent may affect the test results. For example, improper storage and transportation, sample collection, sample processing and test process are not standardized. Please strictly follow the instructions.
6.Due to the characteristics of swab and other sample collection process and viral infection process itself, false negative results may be caused by insufficient sample volume, which should be combined with other clinical diagnosis and treatment information for comprehensive judgment, retest when necessary.
7. After the addition of the sample in the tube the resulting solution is to be considered potentially biohazardous, handle the reagent with appropriate precautions and good laboratory practice.
8. The safe disposal of the reagents supplied must be carried out according to the instruction contained in the specific Safety Data Sheets and in compliance with the national regulations on disposal of potentially hazardous waste.
9. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established; If you have any questions about the test or the results, please contact Sansure`s customer service hotline +86-731-88883176-6116 or send an email to info@sansure.com.cn/ support@sansure.com.cn.

**IVD storage, operating conditions and stability**

The diagnostic kit should be stored in a sealed pouch at -25°C to - 15°C and protected from light. The shelf life of the kit is 12 months. The freeze/thaw cycles should not exceed five, and it keeps stable and valid within 5 days during transportation in a sealed foam box with ice. Please see the date of manufacture and expiry date on the outer package.

**Instrumentation**

The kit is compatible to Fluorescence Quantitative Analysis System containing FAM, HEX/VIC, ROX and CY5 channels such as:

1. Applied Biosystems/7500 Real-Time PCR System
2. Bioer/QuantGene 9600 Fluorescent Quantitative Detection System (Model: S-Q96C)
3. Hongshi/SLAN®-96P Real-Time PCR System
4. Molarray/Real-Time Quantitative Thermal Cycler (Model: MA-6000 )
5. Sansure/Portable Molecular Diagnostic System (S-Q37A/S-Q37B)

**Collecting and preparing specimens**

1. Applicable specimen type: throat swab.
2. Collection of specimen:

Collect throat swab specimens within 3 days after the attack of the disease. Use a specific cotton swab to wipe the postpharyngeal and bilateral tonsils while avoid touching the tongue. Immediately put the throat swab in a 15 mL collection tube with outer screw containing 3-5 mL preservation solution (cell maintenance medium with 5% bovine serum or physiological saline, the former is preferred). Break the swab rod near the tip. Close and tighten the lid then seal it to avoid being dried. Label the tube with a unique identification number.

3. Storage and delivery of specimens:

Samples to be tested can be immediately processed, samples to be tested within 24 hours can be stored at 4°C. Samples that cannot be detected within 24 hours should be stored at -70°C or below (in the absence of -70°C storage conditions, samples to be tested can be stored at -20°C for 10 days, nucleic acid can be stored at -25°C to -15°C for 15 days). Multiple freeze/thaw cycles should be avoided. Samples should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice. The inactivation of samples at 56°C for 30min will not affect the detection of this kit.

**Test procedure**

**1. Preparation of reagent (performed at "reagent preparation region")**

1.1 Take out each component from the diagnostic kit and place them at room temperature. Allow the reagents to equilibrate at room temperature, and then vortex each of them respectively for future use .

1.2 According to the quantity of test specimens, EV/CA16/EV71-Negative Control and EV/CA16/EV71-Positive Control, pipette appropriate quantity of EV/CA16/EV71-PCR Mix and EV/CA16/EV71-Enzyme Mix (If use Sansure's magnetic bead technology to extract nucleic acid, it is recommended to follow the proportion: EV/CA16/EV71-PCR Mix 38 μL/test + EV/CA16/EV71-Enzyme Mix 2 μL/test. Otherwise, use the following one: EV/CA16/EV71-PCR Mix 38 μL/test + EV/CA16/EV71-Enzyme Mix 2 μ L/test + EV/CA16/EV71-Internal Control 0.1 μL/test). Mix them thoroughly and centrifuge it instantaneously for future use .

- Using Sansure Biotech's magnetic bead technology

| | 1 sample | 10 samples | 24 samples |
|---|---|---|---|
| EV/CA16/EV71-PCR Mix (μL) | 38 | 380 | 912 |
| EV/CA16/EV71-Enzyme Mix (μL) | 2 | 20 | 48 |

Note: The above configuration is just for your reference and more to ensure enough volume of the PCR- Mastermix, volume of the actual pipetting may be required.

- Not using Sansure Biotech's magnetic bead technology

| | 1 sample | 10 samples | 24 samples |
|---|---|---|---|
| EV/CA16/EV71-PCR Mix (μL) | 38 | 380 | 912 |
| EV/CA16/EV71-Enzyme Mix (μL) | 2 | 20 | 48 |
| EV/CA16/EV71-Internal Control (μL) | 0.1 | 1 | 2.4 |

Note: The above configuration is just for your reference and to ensure enough volume of the PCR- Mastermix, more volume of the actual pipetting may be required.

1.3 Transfer the above-prepared reagents to the "sample processing room" for later use.

**2. Processing and loading of specimens (performed at "specimen processing region")**

2. 1 Pretreatment of specimens

Add 1 mL of sterile normal saline into the sample collection tube and vortex it thoroughly. Put all the liquids (sample eluent) into a 1.5 mL sterile centrifuge tube (put the cotton swab on the tube wall and press it and then discard) for future use.

2.2 Processing of specimens

Add 200 μL of test specimen, EV/CA16/EV71-Negative Control and EV/CA16/EV71-Positive Control respectively into each tube. Use Sansure's Nucleic Acid Extraction or Purification Reagent to extract the nucleic acid as per the product manual . The EV/CA16/EV71-Internal Control should be put into the Extraction Solution 1 of Nucleic Acid Extraction or Purification Reagent for the extraction and the quantity is 1.0 μL/test.

2.3 Loading of specimens

2.3. 1 Add 40 μL of PCR-Mastermix into each reaction tube according to the quantity of test specimens, EV/CA16/EV71-Negative Control and EV/CA16/EV71-Positive Control.

2.3.2 Pipette respectively 10 μL of processed RNA, EV/CA16/EV71-Negative Control and EV/CA16/EV71-Positive Control into PCR-Mastermix (pipette the liquid up and down and then absorb). Cover the tubes and flip them to remove the bubbles if exist. Centrifuge the tubes at 2000 rpm at 10 seconds or keep flipping the tubes until there are no obvious liquid drops on the tube wall.

**3. PCR Amplification (performed at "amplification and analysis region")** (Refer to user manual of each instrument to adjust the settings.)

3. 1 Place PCR reaction tubes into the specimen wells of the amplification equipment . Set up the EV/CA16/EV71-Negative Control, EV/CA16/EV71-Positive Control and unknown samples in the corresponding sequence and input sample information.

3.2 Select PCR test channel:

a) Select FAM channel (Reporter: FAM, Quencher: None) to test CA16-RNA .
b) Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None) to test EV71-RNA.
c) Select CY5 channel (Reporter: CY5, Quencher: None) to test EV-RNA.
d) Select ROX channel (Reporter: ROX, Quencher: None) to test internal control.
e) Set Passive Reference: none.
f) Set Sample Volume: 50.

3.3 Set cycle parameters (the time parameter varies according to instruments):

| | Step | Temperature | Time | Cycle No. |
|---|---|---|---|---|
| 1 | Reverse transcription | 50°C | 30 min. | 1 |
| 2 | cDNA pre-denaturation | 95°C | 1 min. | 1 |
| 3 | Denaturation | 95°C | 15 sec. | 45 |
| | Annealing, extension, fluorescence collection | 55°C | 30 sec. | |
| 4 | Device cooling | 25°C | 10 sec. | 1 |

**Note:** Due to ABI 7500's technical specification, it can not be set at 30 sec., but at 31 sec. or 32 sec.

When the settings are completed, save the settings and carry out the reaction procedure.

**4. Please process according to the following steps for Portable Molecular Diagnostic System (S-Q37A/S-Q37B):**

**4.1 Pre-run preparation**

4.1.1 Load the amplification reagent component assembly into the extraction reagent component (Nucleic Acid Extraction-Purification Kit, Reference Number: S50016E-12A) to compose the test reagent cartridge;

4.1.2 Open the seal plug of the sample loading hole, add 350μL sample or EV/CA16/EV71-Positive Control or EV/CA16/EV71-Negative Control into the sample loading hole (To ensure Diagnostic System have 300μL samples for nucleic acid extraction); or use transfer pipet from the extraction reagent kit to pipette sample into the sample loading hole (When sample enter the lower bubble of transfer pipet indicates enough sample has been taken). Then close the seal plug.

**4.2 Test Procedure**

4.2.1 Click the "Specimen" button on the instrument display screen to open the door of the instrument and enter the new experiment task setting interface.

4.2.2 Put the prepared consumables into the designated position of the instrument.

4.2.3 Enter specimen information, select the required experimental project in the drop-down menu of Experimental project, enter the corresponding task name in the Task Name bar, and input and select other items that should be input or selected.

4.2.4 Click "Submit" to submit the experimental task and "OK" to run the instrument.

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**Reading test results**

**1. Result Analysis** (Refer to user manual of instrument to adjust the settings.)

Results will be saved automatically when reactions are completed. Adjust Start, End and Threshold values of Baseline of the graph according to analysis result (Users can adjust the values according to the actual situation. Start value can be set between 3- 15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "Analyze" to implement the analysis and make sure each parameter satisfy the requirements given in "5. Quality Control" . Go to "Plate" window to record Ct value.

**2. Quality Control**

The test result is treated as valid if all the conditions in the table below are met for the same test. Otherwise the test result is treated as invalid and needs to be re-tested.

| | EV/CA16/EV71-Negative Control | EV/CA16/EV71-Positive Control |
|---|---|---|
| Ct value | No Ct value display on the channel FAM, HEX/VIC and CY5; positive and Ct ≤ 36 on the channel ROX. | Typical S-shape amplification curve and Ct ≤ 36 on the channel FAM, HEX/VIC and CY5. |

**Reference Range**

Through the research on reference values, the Ct reference value of target gene is determined to be 36, and the Ct reference value of internal control is determined to be 36.

**Interpretation of test results**

Please determine the results of specimens according to the table as below:

| FAM channel | HEX/VIC channel | CY5 channel | ROX channel | Results determination |
|---|---|---|---|---|
| Ct≤36 | Ct>36 or No Ct | Ct≤36 | Ct≤45 or No Ct | Coxsackievirus A16 positive, Enterovirus 71 negative, Enterovirus positive |
| Ct>36 or No Ct | Ct≤36 | Ct≤36 | Ct≤45 or No Ct | Coxsackievirus A16 negative, Enterovirus 71 positive, Enterovirus positive |
| Ct>36 or No Ct | Ct>36 or No Ct | Ct≤36 | Ct≤45 or No Ct | Coxsackievirus A16 negative, Enterovirus 71 negative, Enterovirus positive |
| Ct≤36 | Ct≤36 | Ct≤36 | Ct≤45 or No Ct | Coxsackievirus A16 positive, Enterovirus 71 positive, Enterovirus positive |
| Ct>36 or No Ct | Ct>36 or No Ct | Ct>36 or No Ct | Ct≤36 | Coxsackievirus A16 negative, Enterovirus 71 negative, Enterovirus negative |
| Ct>36 or No Ct | Ct>36 or No Ct | Ct>36 or No Ct | Ct>36 or No Ct | The PCR reaction fails, as there may be inhibitors in the reaction. It is suggested to dilute the RNA by 10 or 100 times and then to retest. If there are fluorescence signals, please determine the results as the above specified in this table, otherwise please collect new specimens and test again. |

**Limitations of the procedure**

1. Test results of the kit can be used only for clinical reference. The symptoms and physical signs, disease history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered during their clinical diagnosis and treatment.

2.The possible reasons for false negative results:

2.1 The unsuitable collection of specimen, delivery, processing and sample in low concentrations may lead to false negative results.

2.2 A mutation in the target sequence to be measured or a change in the sequence due to other causes may lead to false negative results.

2.3 The unreasonable storage of reagent may lead to false negative results.

2.4 Unverified interferences or PCR inhibitors may lead to false negative results.

2.5 Cross-contamination occurring in the sample processing may lead to false positive results.

2.6 The clinical laboratory should be equipped with instruments and operators in strict accordance with relevant requirements outlined in local, state and national regulations. Operate in strict accordance with the product manual.

**Performance characteristics**

When the detection kit is used to detect the 8-tube enterprise positive reference and 8-tube enterprise negative reference, the consistency rate for both positive and negative is 100%.

During the precision testing on the precision references with the concentration of 4.00E+05 copies/mL and 4.00E+03 copies/mL by 10 times respectively, the coefficient of variation of Ct value of the test result is ≤ 5%.

During the detection limitation testing on the detection limitation references with the concentration of 1.00E+03 copies/mL by 20 times, the result is positive within 17 tests or more and the lowest detection limit is 1.00E+03 copies/mL.

The interference test shows that it will not affect the test result determination when the specimens include endogenous or exogenous interferent, such as hemoglobin 2 g/L, respiratory tract mucus 10%, acyclovir 0.2 g/L, ganciclovir 0.2 g/L, ribavirin 0.2 g/L and smecta 0.2 g/L. There is no crosss-reaction with other viruses of the same infection site or similar symptoms of infections, like adenovirus, influenza virus, Epstein-Barr virus, human cytomegalovirus, mycoplasma pneumonia, ureaplasma urealyticum, mycobacterium tuberculosis, staphylococcus aureus, pseudomonas aeruginosa and candida albicans.

**List of references**


1. Development of a multiplex polymerase chain reaction assay for simultaneous identification of human enterovirus 71 and coxsackievirus A16. Journal of Virological Methods, 2010.
2. The largest outbreak of hand; foot and mouth disease in Singapore in 2008: the role of enterovirus 71 and coxsackievirus A strains. International Journal Of Infectious Diseases, 2010.
3. Development of multiplex real-time hybridization probe reverse transcriptase polymerase chain reaction for specific detection and differentiation of Enterovirus 71 and Coxsackievirus A16. DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE, 2008.
4. Guide for prevention and control of hand, foot and mouth disease. Ministry of Health of China, 2008.


**Symbol key**

| Symbols | Meanings | Symbols | Meanings |
|---|---|---|---|
| IVD | *In Vitro* Diagnostic Medical Device | LOT | Batch Code |
| | Use-by date | REF | Reference Number |
| | Manufacturer | | Date of Manufacture |
| | Contains sufficient for <n> tests | | Temperature Limit |
| | Caution | | Consult Instructions for Use |
| PAP | PAP21: Not corrugated cardboard | VERSION | Version |
| PCR MIX | PCR Mix | ENZYME MIX | Enzyme Mix |
| CONTROL − | Negative Control | CONTROL + | Positive Control |
| INTER CONTROL | Internal Control | | Keep away from light |
| | Do not re-use | PRE | Prepackaging |
| EC REP | Authorized representative in the European Community | CE | This product fulfills the requirements of the European Directive 98/79/EC for *in vitro* diagnostic medical devices. |

**Contact information**


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