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

Igenesis CE IVD

REF 106-0053-01

Product Name
Coxsackievirus A16/ Enterovirus 71/Enterovirus RNA Diagnostic Kit(PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
The kit uses real-time multiple fluorescent PCR technology, combined with an Automated Fully Enclosed qPCR Instrument, for the qualitative detection of Coxsackievirus A16 (CA16), enterovirus 71 (Enterovirus 71, EV71) and enterovirus universal virus (Enterovirus, EVG) including Coxsackievirus group A types 2, 4, 5, 6, 7, 9, 10, 12,16, etc., Coxsackievirus group B types 1, 2, 3, 4, 5, etc., Enterovirus 71, Echovirus, etc.The test results can be used for auxiliary diagnosis of hand, foot and mouth disease.

The hand, foot and mouth disease is a common childhood disease caused by enteroviruses. It can be transmitted through the gastrointestinal tract (feces-oral route) or through the respiratory tract (droplets, sneezing, coughing, etc.). The susceptible population is mainly children aged 5 years and below, especially children aged 3 years and below has the highest incidence. Most patients will have fever, skin rashes and ulcers in the hands, feet, mouth, etc., a few patients may have fatal complications such as meningitis, encephalitis, myocarditis, pneumonia, and severe cases can lead to death. The enteroviruses that cause hand-foot-and-mouth disease are more common with CA16 and EV 71.The methods of laboratory diagnostic are mainly include virus isolation, nucleic acid detection, etc.

The kit uses molecular biology methods and requires experimental operators to have received professional training in gene amplification or molecular biology method detection, have relevant experimental operating qualifications and the laboratory should have reasonable biosafety facilities and protective procedures.

Test Principle
The Automated Fully Enclosed qPCR Instrument, uses iCassette technology to automatically perform nucleic acid extraction and nucleic acid amplification. The instrument includes two parts: nucleic acid extraction and multiple fluorescent PCR. At the same time, the built-in QR code scanner can automatically identify the execution process corresponding to this kit. The uniquely designed software is used to execute the extraction and PCR process of the kit, display the results and analyze the results. The kit contains 12 disposable CA16/EV71/EVG iCassettes. The reagents stored in the iCassette include nucleic acid extraction reagents and PCR reaction reagents which are suitable for Automated Fully Enclosed qPCR Instrument. Since the machine is fully enclosed and automatically performs nucleic acid extraction and PCR reactions, it reduces direct cross-contamination of samples. For a complete description of the instrument, please refer to the user manual of the corresponding instrument.

The kit includes nucleic acid extraction reagents and PCR reaction reagents. The nucleic acid extraction reagents contain internal standards, which are used to monitor the entire process of nucleic acid extraction, confirm whether the sample is adequately processed and monitor whether there are PCR inhibitors to avoid PCR false negatives. At the same time, before the start of the PCR reaction, the Automated Fully Enclosed qPCR Instrument will first test the fluorescent signal of the probe to monitor whether the PCR reagents are fully reconstituted, whether the samples are added to the PCR reaction tube and the integrity and stability of the probe.

This kit uses the VP1 region of CA16, the VP1 region of EV71 and the 5'UTR region of Enterovirus as the detection target area (the CA16 virus is labeled with HEX, EV71 is labeled with FAM, EVG is labeled with ROX, and internal control is labeled with CY5). The kit is designed with specific primers and fluorescent probes to perform one-step RT-PCR which can simultaneously detect C A16, EV71 and EVG in one PCR reaction tube.

Components

Kit Components			Content	Quantity
CA16/EV71/EVG iCassette (12 pcs)	Nucleic acid extraction reagent	Magnetic beads	Magnetic microspheres	11μL/iCassette
		CA16/EV71/EVG lyophillization A	Proteinase K	1pc/iCassette
		CA16/EV71/EVG lyophillization B	Pseudovirus lyophillization contains internal control fragments	1pc/iCassette
		Virus lysate solution	Guanidine isothiocyanate	450μL/iCassette
		Virus binding solution	Guanidine isothiocyanate	175μL/iCassette
		Virus washing solution	Sodium chloride	900μL/iCassette
		Virus elution	Tris-HCl	100μL/iCassette
		CA16/EV71/EVG PCR cosolvent solution	PCR Buffer, $MgCl_2$	35μL/iCassette
	CA16/EV71/EVG PCR reagent	CA16/EV71/EVG lyophillization	Specific primer probe, dNTP, enzyme	1pc/tube
Control	CA16/EV71/EVG positive control		Pseudovirus containing target fragment	1 tube (200μL)
	CA16/EV71/EVG negative control		Sterilizing purified water	1 tube (200μL)

Note:Components in kits with different batch numbers are not interchangeable

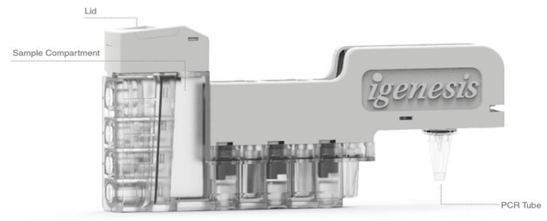

Figure 1 CA16/EV71/EVG iCassette (Lateral View)

Storage condition and Shelf life
1. The kit can be stored at 2-8℃ and the shelf life is 9 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette lid before adding the sample. If you open the iCassette lid, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument: Galaxy Nano, Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Lite or Galaxy Pro.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Timer.
- Pipettes.
- Sterile pipette tips.

Sample Requirements
1. Applicable sample types: oropharyngeal swab
2. Sample collection
2.1 Sampling equipment requirements
2.1.1.Swab: The head used to sample should be the synthetic fiber (eg, polyester fiber), and use aluminum or plastic as a rod.
2.1.2. Sampling tube: Contains 3mL physiological saline or sampling liquid (containing protein stabilizers, antibiotics, buffer to prevent bacteria and fungal growth).
2.2. Sample collection: Use a swab to wipe the posterior pharyngeal wall and tonsils on both sides with moderate force, avoid touching the tongue; quickly put the swab into a sampling tube for storage.
3. Sample storage and transportation
All collected respiratory samples should be airtight and sent for inspection immediately, and stored at 2–8°C for testing within 12 hours, or placed at -20±5°C as soon as possible, but not longer than 1 month.
4. Principles of Biosafety Protection
All operations should comply with local relevant laws and regulations.

Test Method and Operation
1. Prepare CA16/EV71/EVG iCassette
1.1Processing samples or control materials in the samples preparation room,first vortex the collection tube or controls for 10-15 seconds.
1.2 Open the package of CA16/EV71/EVG lyophilization, observe whether the CA16/EV71/EVG lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the CA16/EV71/EVG lyophilization, and make sure the PCR tube is screwed up.
1.3 Open the lid of the iCassette, and pipette 200 μl of sample or controls to the CA16/EV71/EVG iCassette sample compartment as shown in Figure 1. slowly, close the lid tightly.(Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)
1.4 Place the iCassette into the instrument.

2. Test Operation
2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.
2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.
2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.
2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the corresponding channel position of the control software while loading the iCassette)
2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.
2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.
2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.
2.8 After the amplification is completed, the compartment door will open automatically.
For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis
After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
The results of positive control are positive in all four channels (FAM, HEX, ROX and Cy5).
The results of negative control are negative in three channels (FAM, HEX and ROX), Cy5 channel is positive.
Internal control: The CY5 channel is positive (the internal control is specifically competing with the sample. When the sample is positive, the internal control may be tested negative. When the sample is negative, the internal control must be positive. Otherwise it may be a sampling failure or test failure).
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.

Reference Interval
Based on the analysis of clinical sample test results, using the ROC curve method, the final determined Ct positive threshold value for this reagent kit is 36.

Interpretation of Test Results
The sample to be tested will be determined according to the standards in the table below：

FAM Channel	HEX Channel	ROX Channel	CY5 Channel	Results
√	×	√	√/×	EV71 positive or EV71 mixed EVG positive (non-CA16) (Figure 2)
×	√	√	√/×	CA16 positive or CA16 mixed EVG positive (non-EV71) (Figure 3)
×	×	√	√/×	EVG positive(non-EV71 and non-CA16)(Figure 4)
√	√	√	√/×	Positive for EV71, CA16 or EV71, CA16 and mixed EVG(Figure 5)

×	×	×	√	Negative(Figure 6)
×	×	×	×	Invalid
√	√	√	√	The results of positive control.
×	×	×	√	The results of negative control.

Note：“√”indicates the result "has an obvious logarithmic amplification curve and the Ct≤36"；“×”indicates the result "No logarithmic amplification curve or Ct>36".

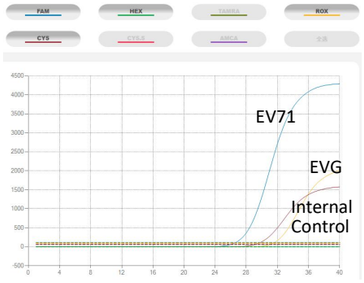

Figure 2 EV71 positive or EV71 mixed EVG positive(non-CA16)

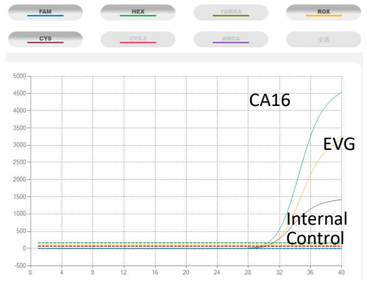

Figure 3 CA16 positive or CA16 mixed EVG positive (non-EV71)

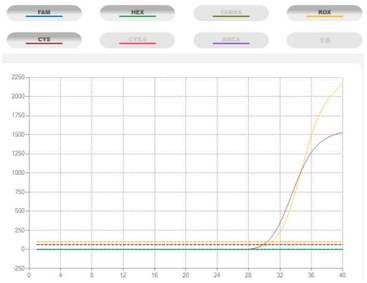

Figure 4 EVG positive,non-EV71, non-CA16

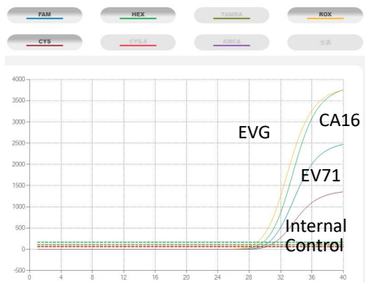

Figure 5 EV71, CA16 positive or EV71, CA16 and EVG mixed positive

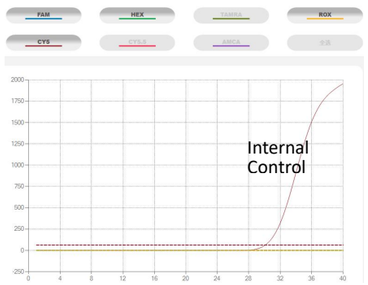

Figure 6 Negative sample

Limitations of test methods

1. The test results of this reagent should be combined with the patient's clinical symptoms and other relevant medical examination results for comprehensive analysis, and should not be used as a basis for patient management alone.
2. Unreasonable sample collection, transportation and processing, as well as improper experimental operation and experimental environment may lead to false negative or false positive results.
3. Variations in the target sequence of the enterovirus or sequence changes caused by other reasons may cause false negative results.
4. Because the positive rates of samples from different stages of the disease are inconsistent, the most suitable sample type for detection and the best sampling time after infection may not be confirmed. Therefore, collecting samples from the same patient multiple times will reduce the possibility of false negative results.

Product Performance Index

1. Analysis sensitivity

S1-S4 are derived from virus strain samples which are serially diluted with RNA virus diluent and calibrated (see the table below for details).

Reference number	Type	Cont: copies/mL
S1	CA6	1.0×10^3
S2	CA10	1.0×10^3
S3	CA16	1.0×10^3
S4	EV71	1.0×10^3

The test results of the 4 sensitivity reference products are all positive and the coincidence rate was 100%.

2. Analysis accuracy

P1-P4 are derived from virus strain samples which are serially diluted with RNA virus diluent and calibrated (see the table below for details).

Reference number	Type	Cont: copies/mL
P1	CA6	1.0×10^4
P2	CA10	1.0×10^4
P3	CA16	1.0×10^4
P4	EV71	1.0×10^4

The test results of the 4 sensitivity reference products are all positive and the coincidence rate was 100%.

3. Analysis specificity

The following conclusions are drawn after the reagent specificity test：Pathogenic microorganisms with the same site of infection or similar symptoms of infection (EB virus, rubella virus, measles virus, influenza A virus, parainfluenza virus, influenza B virus, cytomegalovirus, respiratory syncytial virus, mumps virus, *Klebsiella pneumoniae, Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus, Salmonella, Shigella, Haemophilus influenzae, Pseudomonas aeruginosa* and human spore virus) have no cross reaction

4. Precision

The coefficient of variation of intra-assay precision is less than or equal to 5%.

Precautions

1. The kit must be used within the shelf life.
2. Do not open the iCassette lid before use. If you open it, you must use it within 30 minutes.
3. If you find that the iCassette is oscillated and leaked after adding the sample, do not continue to use it.
4. Prevent sample contamination; experiment personnel should be well protected and wear disposable gloves and masks.
5. Each iCassette is single-test, please do not reuse it.
6. In order to avoid any potential biological hazards in the sample, the test sample should be regarded as an infectious substance and avoid contact with the skin and mucous membranes; sample manipulation and handling must comply with relevant local regulatory requirements
7. This kit is only used for in vitro diagnosis.

References

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Instruction Version

Version: A/1
Date of Issue: May,2019

Symbols

The following symbols may appear on the product labeling:

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
Σ	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
REF	Catalogue number	CE	CE marking of conformity
EC REP	Authorized representative in the European Community		

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