Encephalitis and Meningitis Syndrome 11-Pathogen Nucleic Acid Detection Kit (PCR Melting Curve Method)

Igenesis CE IVD

FOR PROFESSIONAL USE ONLY

REF 106-0149-01

Product Name

Encephalitis and Meningitis Syndrome 11-Pathogen Nucleic Acid Detection Kit (PCR Melting Curve Method)

Specification

12 reactions/kit

Intended Use

This kit is used for typing and differential detection of 11 pathogens of encephalitis and meningitis in samples such as cerebrospinal fluid and whole blood, including ***Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae, Group A streptococcus, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Streptococcus agalactia, Streptococcus suis, Mycobacterium tuberculosis and Cryptococcus.***

Test Principle

The kit uses iCassette technology in combination with supporting instruments to automatically perform nucleic acid extraction and nucleic acid amplification by the instrument throughout the entire process, reducing direct cross-contamination of samples. At the same time, a built-in QR code scanner can automatically identify the execution process corresponding to this kit. A uniquely designed software of the kit is used to perform nucleic acid extraction, whole-process PCR, result display and analysis.

This kit uses one reaction tube to detect 11 different target genes of 11 encephalitis and meningitis pathogens in the sample and determine the specific type of the pathogen in the sample. This kit uses multiplex PCR combined with melting curve analysis technology, and designs primers and probes based on the specific genes of the pathogen. Since each pathogen corresponds to a specific melting curve peak Tm value, pathogen typing and identification detection can be achieved.

Components

Kit Components		Content	Quantity
MES11 iCassette	MES11 iCassette	Guanidine Hydrochloride, Guanidine isothiocyanate, Tris-HCl, Magnetic microsphere	12 pcs
	MES11 lyophillization	Specific primer probe, dNTP, enzyme	12 pcs
Control materials	Sterile Deionized Water	Sterile Deionized Water	2 x 28mL/bottle
	MES11 Positive Control	Plasmid	1 tube
	MES11 Negative Control	Sterilized purified water	1 tube(1200μL)

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

Storage condition and Shelf life

1. The kit can be stored at 2-8℃ and the shelf life is 12 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette cover before adding the sample. If you open the iCassette cover, it should be used within 30 minutes.

Applicable instruments

Automated Fully Enclosed qPCR Instrument: Galaxy Neo, Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided

- Automated Fully Enclosed qPCR Instrument: Galaxy Neo, Galaxy Lite or Galaxy Pro.
- Ultrosonic machine: Galaxy USL III *(not required for Galaxy Neo)*.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Vortex instrument.
- Timer
- Centrifuge
- Pipettes.
- Sterile pipette tips.

Sample Requirements

1. Sample types: cerebrospinal fluid, whole blood.
2. Sample collection

2.1 Cerebrospinal fluid sample: Ensure sterile equipment, informed consent, and proper positioning (usually lateral decubitus or sitting position), locate the lumbar puncture site, typically between L3-L4 or L4-L5 vertebrae, clean the area with antiseptic and apply sterile drapes, insert a lumbar puncture needle with a stylet into the subarachnoid space, remove the stylet and collect cerebrospinal fluid into sterile tubes.

2.2 Whole blood sample: Gather sterile equipment, identify a vein, typically in the arm, clean the site with an alcohol swab, apply a tourniquet, insert the needle, and draw blood into an anticoagulant tube (e.g., EDTA), label the sample with patient information and send it for analysis.

3. Sample storage and transportation

The specimens used for virus isolation and nucleic acid detection should be tested as soon as possible.All collected samples can be tested immediately, or stored at 2–8 °C for 7 days, or stored at ≤ −20 °C for 6 months, avoiding repeated freezing and thawing.

4. Principles of Biosafety Protection:

All operations should comply with relevant national laws and regulations.

Test Method and Operation

1. Prepare the MES11 iCassette

Open the package of MES11lyophilization, observe whether the MES11 lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the MES11 lyophilization, and make sure the PCR tube is screwed up.

2. Process the samples

2.1 Samples pretreatment

2.1.1 For cerebrospinal fluid

No need for pretreatment.

2.1.2 For whole blood sample

No need for pretreatment.

2.2 Ultrasonic Crushing

Pipette 1mL of the cerebrospinal fluid sample, or 50ul of whole blood sample and 950ul of sterile deionized water and slowly add it to the MES11iCassette sample compartment as shown in Figure 1, close the lid tightly, put it into the ultrasonic machine, and sonicate for 20 seconds. (Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes). Place the ultrasonicated iCassette into the instrument.

Note: For the Galaxy Neo, the ultrasound functionality is already built-in, so simply add the sample to be tested into the iCassette and insert it into the machine.

2.3 Process negative and positive controls

Pipette 600μL of sterile saline or sterile deionized water to re-dissolve the MES11 positive control, then take 600μL of MES11 positive control or negative control slowly add it to the MES11iCassette sample compartment as shown in Figure 1, close the lid tightly. Place the iCassette into the instrument.

Figure 1. MES11 iCassette (Lateral View)

3. Test Operation

3.1 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.

3.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.

3.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.

3.4 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)

3.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.

3.6 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.

3.7 After the program starts, the progress of the instrument running will be displayed in the main interface.

3.8 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.

4. Result Analysis

After the experiment is finished, the instrument will automatically save the results, and output the Tm value and melting curves with results interpretation in the interface.

Results Interpretation

The Tm reference distribution for 11 encephalitis and meningitis pathogens in each channel are as follows:

Channels	Pathogen	Tm value reference range (℃)
FAM	*Escherichia coli*	60.0-64.0
	Mycobacterium tuberculosis	66.0-69.0
	Staphylococcus aureus	71.0-75.0
	Cryptococcus	76.0-80.0
CY5	*Listeria monocytogenes*	66.0-69.5
	Haemophilus influenzae	70.0-74.0
ROX/Texas Red	*Streptococcus agalactiae*	59.0-63.0
	Group A streptococcus	63.5-67.0
	Streptococcus pneumoniae	68.0-72.0
	Streptococcus suis	73.5-77.0
VIC/HEX	RNase P	71.0-75.0
	Neisseria meningitidis	76.0-80.0

Quality Control

Internal control: When testing human samples, the internal control RNase P in the VIC /HEX channel must have a specific melting peak;

The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.

Test results of positive control: The pathogen types corresponding to melting curve analysis of FAM, CY5, ROX/Texas Red, and VIC/HEX are *Cryptococcus, Haemophilus influenzae, Streptococcus suis* and *Neisseria meningitidis*, respectively.

Test results of negative control:Melting curve analysis, the results were all negative.

Limitations of Test Method

1. The test results are related to the sample collection, transportation and storage conditions. Any mistakes will lead to false negative results. If the sample is cross-contaminated during the processing, a false positive result may occur. The test results are for clinical reference only and cannot be used alone as a basis for confirming or excluding cases.
2. This kit detects the conserved region of the pathogen, which rarely mutates, but it does not rule out the possibility that genetic mutations of the pathogen during the epidemic may lead to false negative results.

Product Performance Index

1. The LoD of the kit: 500 copies/mL.
2. Specificity: There is no cross-reaction with other common pathogens with the same infection site or similar infection symptoms.
3. Precision: The coefficient of variation of the test precision reference is less than 5%.

Precautions

1. If the iCassette is oscillated after adding the sample, do not use the iCassette.
2. Clinical laboratories should strictly follow the management standards in the local related regulations for molecular biology laboratories and clinical gene amplification laboratories.
3. Each iCassette is single-use, please do not reuse it.
4. In the sample processing stage, use a negative pressure ultra-clean workbench.
5. During the experiment, the medical staff must wear work clothes, disposable gloves, and use self-discharging pipettes.
6. After the experiment, the workbench and pipette were treated with 2% sodium hypochlorite or 75% alcohol, and then irradiated with a UV lamp for 30 minutes.

Instruction Version
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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
	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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