Mycoplasma Pneumoniae / Adenovirus / Rhinovirus / Human Metapneumovirus Diagnostic Kit (PCR-Fluorescence)

REF 106-0069-01

Product Name
Mycoplasma Pneumoniae / Adenovirus / Rhinovirus / Human Metapneumovirus Diagnostic Kit (PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
This kit uses real-time multiplex fluorescent PCR technology, combined with Automated Fully Enclosed qPCR Instrument, for in vitro qualitative detection of Mycoplasma pneumoniae, Adenovirus, Rhinovirus, and Human Metapneumovirus nucleic acid in human oropharyngeal swab samples. The test results can be used for auxiliary diagnosis respiratory tract infection.

The incidence rate of acute respiratory infection occupies the first place among all kinds of diseases in children in China and it is a common and frequently-occurring disease in pediatrics. At present, it has been confirmed that more than 80% of them are caused by virus infection, including rhinovirus, human metapneumovirus, adenovirus, etc. Respiratory virus is highly contagious, which can cause local outbreaks and spread around the world, seriously affecting human health. The same virus can cause a variety of clinical symptoms-Mycoplasma pneumoniae (MP) is already one of the common pathogens of community-acquired pneumonia. It mainly causes acute respiratory system diseases in children, and at the same time involves other systemic diseases, and the degree of infection varies greatly. Adenovirus (Adenovirus, Adv) is divided into seven subspecies -A~G, and different genotypes can cause different clinical manifestations, including high fever, cough, sore throat, etc., and a few develop into severe pneumonia. Rhinovirus (RhV) mainly causes the common cold in adults, but also causes bronchial pneumonia, acute asthma and other diseases in children and young children. Epidemiological trends of human metapneumovirus (hMPV) infection vary by region, and two virus lineages may circulate in the same epidemic season. After infection, it can lead to upper and lower respiratory tract infections and wheezing diseases. Children and immunocompromised populations often lead to severe respiratory infections, which can be co-infected with other viruses, while recessive infections are rare. Therefore, the rapid detection of Mycoplasma pneumoniae, human rhinovirus, human metapneumovirus, and adenovirus is beneficial to the diagnosis and treatment of patients.

This kit uses the method of molecular biology for detection. It is required that the experimental operators should have received professional training in gene amplification or molecular biology method detection, have relevant experimental operation qualifications, and the laboratory should have reasonable biosafety facilities and protection procedures.

Test Principle
Automated Fully Enclosed qPCR Instrument uses iCassette to automatically perform nucleic acid extraction, nucleic acid amplification, data reading and result analysis. The instrument includes two parts of nucleic acid extraction and multiplex fluorescent PCR. At the same time, the built-in QR code scanner can automatically identify the execution process corresponding to this kit. The software is used to execute the whole process of extraction and PCR of this kit, display the results and analyze the result. This kit contains 12 disposable MP/ADV/HRV/hMPV iCassette. 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 Automated Fully Enclosed qPCR Instrument is fully enclosed and automatically performs nucleic acid extraction and PCR reactions, the samples' direct cross-contamination is reduced. For a complete description of the instrument, please refer to the corresponding instrument user manual.

This kit uses real-time multiplex fluorescent PCR technology to detect specific genes of Mycoplasma pneumoniae, rhinovirus, human metapneumovirus, and adenovirus, and designs specific primers and fluorescent probes respectively (where Mycoplasma pneumoniae is labeled with FAM, adenovirus Viruses are labeled with TEXAS RED, human metapneumovirus is labeled with CY5, and rhinovirus is labeled with CY5.5), and primers and probes (HEX label) for built-in quality control samples are designed at the same time, and it will be confirmed whether the target virus has been fully processed through the detection of quality control samples, and detected whether there are PCR reaction has the inhibitors.

Components

Kit Components			Content	Quantity
MP/ADV/HRV/hMPV iCassette (12 pcs)	Nucleic acid extraction reagent	MP/ADV/HRV/hMPV lyophilization A	Proteinase K	1 pc/iCassette
		MP/ADV/HRV/hMPV lyophilization B	Pseudovirus lyophilization containing internal standard fragments	1 pc/iCassette
		Virus lysate solution	Guanidine isothiocyanate	450 µl/iCassette
		Virus binding solution	Guanidine isothiocyanate	175 µl/iCassette
		Virus washing solution	Sodium chloride	900 µl/iCassette
		Virus eluent	Tris-HCl	100 µl/iCassette
		Magnetic beads	Magnetic Microspheres	40 µl/iCassette
	MP/Adv/RhV/hMPV PCR reagent	MP/Adv/RhV/hMPV PCR cosolvent solution	PCR Buffer, $MgCl_2$	35 µl/iCassette
		MP/Adv/RhV/hMPV lyophilization	Specific primer probe, dNTP, enzyme	1 pc/tube
Control	MP/ADV/HRV/hMPV positive control		Plasmids or pseudoviruses containing gene fragments of MP, ADV, HRV, hMPV	1tube(200µL)
	MP/ADV/HRV/hMPV negative control		Sterilized purified water	1tube(200µL)

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 9 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 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.
- Vortex instrument.
- 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.
3. Principles of Biosafety Protection
All operations should comply with local relevant laws and regulations.

Test Method and Operation
1. Prepare MP/ADV/HRV/hMPV 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 MP/ADV/HRV/hMPV lyophilization, observe whether the MP/ADV/HRV/hMPV lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the MP/ADV/HRV/hMPV 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 MP/ADV/HRV/hMPV 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.

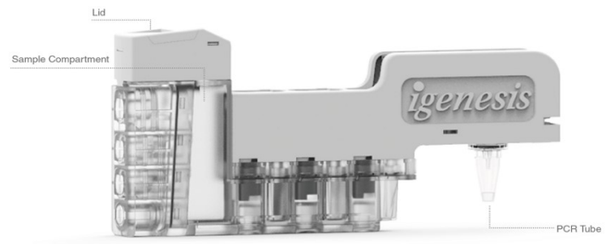

Figure 1 MP/ADV/HRV/hMPV iCassette (Lateral View)

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
Internal control: The effective range of Ct38 in the HEX channel is qualified as the internal standard quality control.

Positive control test results: FAM channel, TEXAS RED channel, CY5 channel, and CY5.5 channel are all positive;

Negative control test results: FAM channel, TEXAS RED channel, CY5 channel, and CY5.5 channel are all negative, and HEX channel is positive.

In the same experiment, the above requirements must be met at the same time, otherwise, the 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 38.

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

FAM Channel	TEXAS RED Channel	Cy5 Channel	Cy5.5 Channel	HEX Channel	Results
√	×	×	×	√	Mycoplasma Pneumoniaepositive(Figure 2)
×	√	×	×	√	Adenovirus positive(Figure 3)
×	×	√	×	√	hMPV positive(see Figure 4)
×	×	×	√	√	Rhinovirus positive(Figure 5)
√	√	√	√	√	Positive control
×	×	×	×	√	Negative (Figure 6)
×	×	×	×	×	Invalid

Note: "v" means that the result "has an obvious logarithmic amplification curve", and Ct ≤38; "×" indicates the result "no logarithmic amplification curve", and Ct >38. The HEX channel is an internal standard channel. Due to the specific competition with the sample, when the test sample is detected as positive, it may be detected as negative.

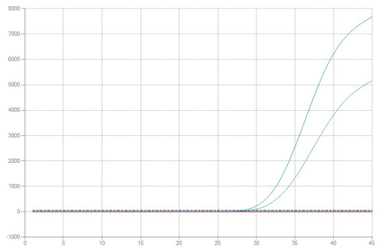

Figure 2. MP Positive

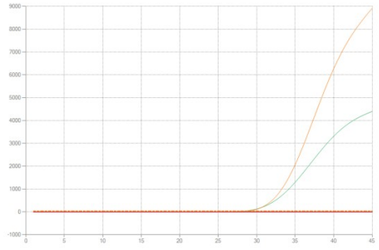

Figure 3.Adenovirus Positive

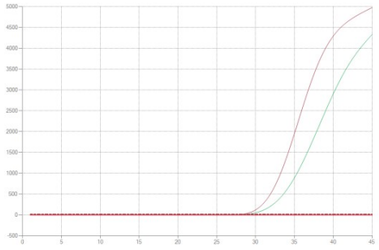

Figure 4. hMPV Positive

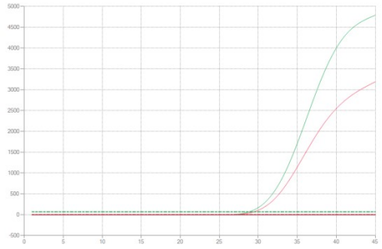

Figure 5.Rhinovirus Positive

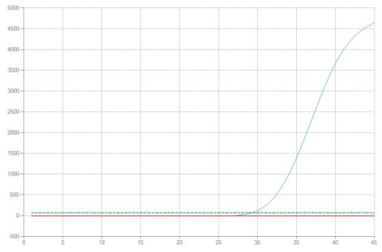

Figure 5. negative sample

Limitations of Test Method

1.The test results of this reagent should be comprehensively analyzed in conjunction with the clinical symptoms of patients and other related medical examination results, and it should not be used as a basis for patient management.
2.If the positive result is adenovirus, further experiments are recommended to confirm the subtype of adenovirus, and consult and negotiate with local public health prevention agencies.
3.Unreasonable sample collection, transportation and processing, low virus titer in the sample, and improper experimental operations and experimental environment may cause false negative results.
4.Variation of the target sequence of Mycoplasma pneumoniae/Adenovirus/Rhinovirus/human Metapneumovirus or sequence changes caused by other reasons may lead to false negative results.
5.During specimen collection, patients who received live attenuated vaccines may result in a positive test result.
6.The positive rates of samples at different stages of different disease courses were not consistent.
7.The nucleic acid sequence to be tested may appear in the body for a long time, regardless of viral activity. A positive nucleic acid test does not necessarily mean that you are currently infected with the corresponding virus or that it is the causative agent of clinical symptoms.
8.Positive and negative predictive values depend largely on prevalence. Test performance for some viruses may vary with test prevalence and test population.

Product Performance Index

1. Accuracy
This kit detects in-house reference material: 13 in-house reference materials (P1-P13) are detected as positive, and 13 sensitivity reference materials (S1-S13) are detected as positive, with a coincidence rate of 100%. The negative in-house reference materials (N1-N7) were detected as negative, and the coincidence rate was 100%.
2. Limit of Detection:

Pathogens	LoD
Mycoplasma Pneumoniae	1× 10^3 copies/mL
Adenovirus	1× 10^3 copies/mL
Rhinovirus	1× 10^3 copies/mL
human Metapneumovirus	1× 10^3 copies/mL

Analysis specificity
The verified pathogenic microorganisms that are the same as the infection site or have similar infection symptoms are negative and have no cross-reaction, and the verified concentrations are as follows:

Microorganism	Concentration	Microorganism	Concentration
Measles Virus	3.2 × 10^8 $TCID_{50}$ /L	Streptococcus Pneumonias	1.62× 10^6 CFU/mL
Escherichia Coli	12～14 × 10^{10} CFU/mL	Bordetella Pertussis	2× 10^7 CFU/mL
Pseudomonas aeruginosa	24～40 × 10^{10} CFU/mL	Chlamydia Pneumoniae	1×10^6 copies/mL
Staphylococcus Aureus	14～22 × 10^{10} CFU/mL	Haemophilus Influenzae	3.5× 10^7 CFU/mL
Mumps Virus	6.3 × 10^8 $TCID_{50}$ /L	Legionella Pneumophila	2× 10^7 CFU/mL
Rubella Virus	3.2 × 10^8 $TCID_{50}$ /L	Citrobacter	2.6× 10^6 CFU/mL
Gonococcus	1× 10^6copies/mL	Klebsiella Pneumoniae	3× 10^7 CFU/mL
Mycobacterium Tuberculosis	1× 10^4 copies/mL	Cryptococcus neoformans	2.1× 10^6 CFU/mL
Parainfluenza-2	1× 10^5 copies/mL	Candida Albicans	2.1× 10^6 CFU/mL
Neisseria Meningitidis	1× 10^6 copies/mL	Enterovirus 71	3.6× 10^6copies/mL
Parainfluenza-3	1× 10^5 copies/mL	Cytomegalovirus	1× 10^6copies/mL
Parainfluenza-1	1× 10^5 copies/mL	Influenza A Virus	1× 10^6 copies/mL
Influenza B Virus	1× 10^6 copies/mL	Respiratory Syncytial Virus	1× 10^6 copies/mL

3. Interfering substances
Endogenous substances: blood (5%), mucin (2mg/mL) do not interfere with the detection of the kit.
Exogenous substances: the sample may contain tobramycin (0.05 mg/mL), oxymetazoline hydrochloride (99 μg), dexamethasone (0.6 mg/L), menthol (13.5 mg/L), mupirocin (20 mg/L), budesonide (2.5 mg/L), oseltamivir (0.15 mg/mL), ribavirin (15 mg/mL), cetirizine hydrochloride (2.5 mg/mL), mometasone (1 g/mL).
All the above specific samples were negative by this kit test, which was in line with the expected setting of the kit.
4. Precision:
Intra-batch precision coefficient of variation (CV%) ≤ 5%.

Precautions

1. Do not use the iCassette if it is subjected to vibrations after adding the sample.
2. Each iCassette is single-used, please do not reuse it.
3.The tips used in the experiment should have reasonable cleaning and quality inspection procedures to avoid false negative results caused by RNase contamination or amplification reaction inhibitors.
4.Sample preparation should be carried out in a biological safety cabinet. During the experiment, wear overalls, disposable gloves, and use a self-unloading pipette.
5.The used tips for sample preparation should be put into a container containing disinfectant. After the preparation experiment is completed, they can be sterilized together with the iCassette before being discarded.
6.After the experiment, the workbench and pipette were treated with 10% hypochlorous acid or 75% alcohol, and then irradiated with ultraviolet light for 30 minutes.

References

1. Jung-Yun H , Hoan-Jong L , Piedra P A , et al. Lower Respiratory Tract Infections due to Adenovirus in Hospitalized Korean Children: Epidemiology, Clinical Features, and Prognosis[J]. Clinical Infectious Diseases(10):10.
2. Abd-Jamil J , Teoh B T , Hassan E H , et al. Molecular identification of adenovirus causing respiratory tract infection in pediatric patients at the University of Malaya Medical Center[J]. BMC Pediatrics, 2010, 10(1):46-46.

Instruction Version

Version: A/0
Date of Issue:May,2022

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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