Mycoplasma Pneumoniae DNA Fluorescence Diagnostic Kit (PCR-Fluorescence Probing)

Product Identification

Product Name: *Mycoplasma Pneumoniae* DNA Fluorescence Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number: S3016E

Package Specification: 48 tests/kit, Pre-packaged 12 tests/kit

Intended Use

The *Mycoplasma Pneumoniae* DNA Fluorescence Diagnostic Kit (PCR-Fluorescence Probing) is an *in vitro* nucleic acid amplification test for the detection of *Mycoplasma pneumoniae* DNA in human sputum and throat swab. It is intended for use as an aid in the diagnosis of an MP infection and providing a molecular-diagnostics-based solution.

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

Test principle summary and explanation

Summary

Mycoplasma pneumoniae (MP) is a pathogenic microorganism. It is mainly transmitted through buccal and nasal mucus and by air causing respiratory diseases, with the highest incidence in children and adolescents. Respiratory infection has the manifestations of pharyngitis and bronchitis, with a few cases causing infection to the lung. Recently, incidence among infants and children is increasing, therefore, early diagnosis and treatment can decrease the exacerbation of acute pneumonia in children.

Test principle

The kit uses a nucleic acid lysis buffer to allow rapid lysis and release of MP-DNA from a sputum specimen or throat swab which has been processed with concentrate. By applying real-time fluorescence PCR technology, this test uses a pair of specific primers which are designed to target a conserved sequence of MP-DNA, and a specific fluorescence probe, accompanied with other components in the PCR mix, to achieve quick detection of MP-DNA through fluorescent signal changes.

The PCR detection system uses UNG enzyme + dUTP contamination-proof system, which can fully degrade possible unwanted amplification products, to avoid a false positive result.

The PCR detection system uses an 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
		48T	Pre-packaged 12T	Pre-packaged 12T (S-Q37A/B)	
1	MP-Lysis Buffer	2.5 mL/tube × 1 tube	20 μL/tube × 12 tubes	2.5 mL/tube × 1 tube	KCl, SDS, Surfactin
2	MP-Enzyme Mix	96 μL/tube × 1 tube	2 μL/tube × 12 tubes	2 μL/tube × 12 tubes	DNA polymerase, UNG enzyme
3	MP-Internal Control	50 μL/tube × 1 tube	1 μL/tube × 12 tubes	1 μL/tube × 12 tubes	Cloned plasmid containing the target gene fragment
4	MP-PCR Mix	912 μL/tube × 2 tubes	38 μL/tube × 12 tubes	38 μL/tube × 12 tubes	Primers, probes, dNTPs, Mg^{2+}, PCR buffer solution
5	MP-Positive Reference A (4.00E+07 copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	1500 μL/tube × 1 tube	Cloned plasmid containing the target gene fragment
6	MP-Positive Reference B (4.00E+06 copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	1500 μL/tube × 1 tube	Cloned plasmid containing the target gene fragment
7	MP-Positive Reference C (4.00E+05 copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	1500 μL/tube × 1 tube	Cloned plasmid containing the target gene fragment
8	MP-Positive Reference D (4.00E+04 copies/mL)	50 μL/tube × 1 tube	50 μL/tube × 1 tube	1500 μL/tube × 1 tube	Cloned plasmid containing the target gene fragment
9	MP-Negative Control	50 μL/tube × 1 tube	50 μL/tube × 1 tube	1500 μL/tube × 1 tube	MP negative specimen (inactivated)
10	MP-Positive Control	50 μL/tube × 1 tube	50 μL/tube × 1 tube	1500 μL/tube × 1 tube	MP positive specimen (inactivated)
11	Concentrate	5 mL/tube × 1 tube	5 mL/tube × 1 tube	5 mL/tube × 1 tube	PEG-6000, NaCl, purified water

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, various models of pipettes and pipette tips (10 μL, 200 μL and 1,000 μL tips with filters are preferred), desktop centrifuge, desktop vortex mixer, magnetic-bead separator.
2. Reagent required but not provided: Cell preservation solution, Nucleic Acid Extraction-Purification Kit (Reference Number: S50016E Series) or Sample Release Reagent (Reference Number: S1012E Series) manufactured by Sansure Biotech Inc.

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. 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 perform quality control for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratories; laboratory personnel must receive professional training; test processes must be performed in separated rooms; all consumables should be for single use only after sterilization; special instruments and devices should be used for every process; all lab devices used in different processes and rooms should not be cross-used.
4. All specimens 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 specimens and waste must meet relevant requirements outlined in local, state and national regulations.
5. Before use, all reagents must be fully thawed at room temperature and mixed thoroughly.
6. If there is no HEX or VIC test channel for fluorescence PCR instrument, monitoring of internal control can be omitted. Do not add internal control at the above step of 1.2 to avoid interference from extra multicolor fluorescence. Please consult with Sansure staff about the option of detection channel.
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

1. The shelf life of the kit is 12 months at -25°C to -15°C and protected from light.
2. Please refer to the date of manufacture and expiry date on the outer package.
3. The reagents keep valid and stable within the expiry date if not used. As long as the container of the reagent is opened, the freeze/thaw cycles should not exceed three.

Instrumentation

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

1. Angilent/AriaDx Real-Time PCR System
2. Applied Biosystems/7500 Real-Time PCR System
3. Bio-Rad/CFX 96 Deep Well Dx ORM
4. Hongshi/SLAN®-96P Real-Time PCR System
5. Molarray/Real-Time Quantitative Thermal Cycler (Model: MA-6000)
6. Roche/LightCycler® 480 instrument II
7. Roche LightCycler® 480 System
8. Sansure/Portable Molecule Workstation (Model: S-Q31A/S-Q31B)
9. Sansure/Portable Molecular Workstation (Model: S-Q36A)
10. Sansure/Portable Molecular Diagnostic System (Model: S-Q37A/S-Q37B)
11. SLAN-96P® Real-Time PCR System
12. Thermofisher QuantStudio™ 5 Real-Time PCR System

Collecting and preparing specimens

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

2.1 Collection of sputum:

2.1.1 Use single-use sputum aspirator.

2.1.2 Place the patient in a horizontal position with the head up.

2.1.3 Put the aspirator's tube slowly and gently in the throat and adjust it to negative-pressure to aspirate steadily the secretion laying in the deep of patients' airways (It is recommended to aspirate atomized sputum). Repeat the aspiration several times. Use the collected sputum as specimen and seal it and send it for detection.

2.1.4 The collected sputum should be sent for detection as soon as possible before it goes dry and becomes invalid for detection.

2.2 Collection of throat swab: use sterile cotton swab to collect secretions in the throat and place the throat swab in a sterile glass tube. Seal it and send it for detection.

3. Storage and delivery of specimens:

Specimens collected via the above-mentioned method can be used for immediate detection, or stored at 2-8°C for up to 24 hours, or below -20°C for a long term storage. Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

Test Method

1. Please process according to the following steps for7500 Real-Time PCR System, SLAN®-96P Real-Time PCR System, Real-Time Quantitative Thermal Cycler (Model: MA-6000), AriaDx Real-Time PCR System, CFX 96 Deep Well Dx ORM, LightCycler® 480 instrument II and QuantStudio™ 5 Real-Time PCR System instrument:

1. 1 Preparation of reagent (performed at "reagent preparation room")

1.1.1 Take all the components out of the kit and equilibrate them to room temperature. Then vortex each component separately for later use.

1.1.2 According to quantity of specimens to be tested, MP-Negative Control and MP-Positive Control, pipette appropriate quantity of MP-PCR Mix, MP-Enzyme Mix and MP-Internal Control (MP-PCR Mix 38 μL/test+ MP-Enzyme Mix 2 μL/test+ MP-Internal Control 0.4 μL/test), fully mix them to make a PCR-Mastermix and centrifuge it instantaneously for later use.

	1 specimen	10 specimens	24 specimens	48 specimens
MP-PCR Mix (μL)	38	380	912	1824
MP-Enzyme Mix (μL)	2	20	48	96
MP-Internal Control (μL)	0.4	4	9.6	19.2

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.

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

2.1 Processing of MP-Negative Control, MP-Positive Control and MP-Positive References

Pipette 10 μL of MP-Negative Control, MP-Positive Control and MP-Positive References A-D respectively and then mix each of them with 10 μL of MP-Lysis Buffer respectively for later use.

2.2 Processing of sputum

Add the normal saline into sputum specimen with the volume of saline two to three times to specimen. Vortex it thoroughly and wait to liquefy the sputum. Pipette 1000 μL of liquefied specimen to a 1.5 mL centrifuge tube (be careful not to pipette solid impurities out). Add 100 μL of concentrate to the centrifuge tube. Vortex it and thoroughly mix it. Centrifuge it at 12,000 rpm for 5 minutes. Discard the supernatant (It is recommended to leave 20 μL of the supernatant in the tube). Add 50 μL of MP-Lysis Buffer into the precipitate and vortex it or pipette it up and down to mix it and then hold it for 10 minutes to allow thorough lysis for later use.

2.3 Processing of throat swab

Add 1 mL of sterile saline into specimen collection tube. Vortex it and mix it thoroughly. Then transfer all the liquids (specimen eluent) to a 1.5 mL centrifuge tube (press to dry the cotton swab against the centrifuge tube wall and then discard the swab). Add 100 μL of concentrate to the centrifuge tube. Vortex it and thoroughly mix it. Centrifuge it at 12,000 rpm for 5 minutes. Discard the supernatant (it is recommended to leave 20 μL of supernatant in the tube). Add 50 μL of MP-Lysis Buffer into the precipitate and vortex it or pipette it up and down to mix it and then hold it for 10 minutes to allow thorough lysis for later use.

2.4 Loading of specimens

2.4.1 Add 10 μL of the above processed test specimen, MP-Negative Control, MP-Positive Control and MP-Positive References A-D respectively into each PCR reaction tube.

2.4.2 Add 40 μL of PCR-Mastermix into each tube. Pipette it up and down for 2-3 times. Cover the tube lid (Remove the bubbles). Centrifuge it at 2000 rpm for 30 seconds.

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

3.1 Place the PCR reaction tube into the specimen wells of the amplification device. Set up the MP-Negative Control, MP-Positive Control, MP-Positive References A-D and unknown samples in the corresponding sequence and input sample information and concentration of MP-Positive References A-D.

3.2 Select PCR test channel

3.2.1 For ABI, Stratagene series:

1) Select FAM channel (Reporter: FAM, Quencher: None) to test MP-DNA.
2). Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None) to test MP-Internal Control.
3). Set passive reference: none.
4). Set sample volume: 50.

3.2.2 For Roche LightCycler 480:

Choose "New Experiment". Click "Dual Color Hydrolysis Probe/ UPL Probe" in the drop-down menu of setup panel-Detection format. Do the following in the drop-down menu of "Customize":

1) Select FAM channel to test MP-DNA;
2) Select VIC/HEX/Yellow 555 channel to test MP-Internal Control.
3) Set reaction volume: 50.

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

3.3.1 ABI (such as SLAN-96P, Life Technologies QuantStudio ™ 5 , MA-6000, CFX96)**, Stratagene series:**

	Step	Temperature	Time	Cycle No.
1	UNG enzyme reaction	50°C	2 min.	1
2	Taq enzyme activation	94°C	5 min.	1
3	Denaturation	94°C	15 sec.	45
	Annealing, extension, fluorescence collection	57°C	30 sec.*	
4	Device cooling(optional)	25°C	10 sec.	1

Note: Due to the device ABI 7500's technical specification, it can not be set at 30 sec. but 31 sec.)

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

3.3.2 LightCycler 480 (choose default value for non-listed parameters)

Program	Target(°C)	Acquisition Mode	Hold (hh:mm:ss)	Cycles	Analysis Mode
1	50	None	00:02:00	1	None
2	94	None	00:02:00	1	None
3	94	None	00:00:05	45	Quantification
	57	Single	00:00:30		
4 (optional)	25	None	00:00:10	1	None

When the settings are completed, save settings, operate the reaction procedure.

3.4 Please process according to the following steps for Portable Molecular Workstation (Model: S-Q36A):

3.4.1 Preparation of consumables and reagents

1) Take out the Consumables kits and reagents.

2) Put Internal Control into the **Well A**; Sample Release Reagent (Reference Number : S1012E Series) into the **Well B**; Put MP-PCR Mix into the **Well C**; Put MP-Enzyme Mix into the **Well D**,(The well location information has been marked on the carrier set)

3) Add 20μL sample to be tested or MP-Positive Control or MP-Negative Control into the **Well B** (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

3.4.2 Test Procedure

1) Click the "and " button on the instrument display screen to open the door of the instrument and put the prepared consumables into the designated position of the instrument.

2) Click the "New" on the instrument display screen to enter the new experiment task setting interface.

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

3.5 Please process according to the following steps for Portable Molecule Workstation (Model: S-Q31A&B):

3.5.1 Preparation of consumables and reagents

1) Take out the reaction tube carrier, PCR reaction tube and Tip;

2) Put the Tip into **Well H**,and PCR reaction tube into **Well** PCR(The **Well** location information has been marked on the reaction tube carrier);

3) Put Internal Control into the **Well A**;Put Sample Release Reagent (Reference Number : S1012E Series) into the **Well B**; Put MP-PCR Mix into the **Well C**; Put MP-Enzyme Mix into the **Well D**;

4) Add 20μL sample to be tested or MP-Positive Control or MP-Negative Control into the **Well B** (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

3.5.2 Test Procedure(Refer to user manual of each instrument to adjust the settings.)

1) Gently press the front door to open it.

2) Place the **Well A** of reagent strip into the instrument towards the outside of the instrument,and close the front door of the instrument.

3) Click the**"Experimental task"** on the instrument display screen to enter the interface of setting new experimental task.

4) 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.

5) Click **"Submit"** to submit the experimental task and **"OK"** to run the instrument and start the experimental task successively.

6) When the Portable Molecule Workstation (Model: S-Q31B) shows **"Please transfer the PCR tube to the 1/2/3/4"**(The S-Q31A shows "Please transfer the PCR tube") on the interface, take out the PCR tube and cover it well, then centrifuge it instantaneously.

7) Insert the PCR tube into the PCR amplification module (the "PCR 1/2/3/4" cover has been automatically opened at this time), close the PCR lid of the amplification module, then click **"OK"** for amplification detection.

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

3.6.1 Pre-run preparation

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;

2) Open the seal plug of the sample loading hole, add 350 μL sample or MP-Positive Control or MP-Negative Control into the sample loading hole (To ensure Diagnostic System have 300 μL 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 indicating enough sample has been taken). Then close the seal plug.

3.6.2 Test Procedure

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.

2) Put the prepared consumables into the designated position of the instrument.

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) Click **"Submit"** to submit the lab task and **"OK"** to run the instrument.

Reading test results

1. Result analysis (Refer to user manual of each instrument to adjust the settings)

When the reactions are completed, results will be saved automatically. After analysis, adjust Start, End and Threshold values of Baseline of the graph (users can adjust them according to the actual situations. Start value can be set at 3-15, end value at 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 satisfies the requirements given in “5. Quality Control”. Go to “Plate” window to record the 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.

	MP-Positive Control	MP-Negative Control	MP-Internal Control	MP-Positive References (A, B, C, D)
Ct value	≤ 30	No Ct	≤ 40	≤ 39

Positive Reference Values

Through the research on reference values, the Ct reference value of target gene is determined to be 39, and the Ct reference value of internal control is determined to be 40.

Interpretation of test results

1. Determination of negative or positive results

Conclusion	Ct value of sample	Ct value of internal control	Amplification curve of sample
Positive	≤ 39	-	like "S"
Negative	No Ct	≤ 40	like "--"
Out of limit (concentration < 4.00E+02 copies/mL)	> 39	≤ 40	like "S"
Invalid*	-	> 40 or No Ct	-

Note: *This suggests an investigation should be carried out to find out the reasons when Ct value of the internal control is > 40 or No Ct and retest it. (If repeated tests still produce invalid results, please contact Sansure Biotech at info@sansure.com.cn)

Limitations of Detection Method

Detection result is related to specimen collection, processing, delivery and storage quality. Any deviation from the stated procedure will lead to an inaccurate detection result. Cross-contamination during specimen processing may also result in a false-positive result.

Product Performance Index

When the kit is used to detect the enterprise's work references, the consistency rate for both negative and positive reaches 100%. Precision test shows excellent reproducibility in both intra-batch and inter-batches with its coefficient of variation of Ct value <10%, and its coefficient of variation of concentration <50%. The sensitivity of this kit is determined to be 4.00E+02 copies/mL. It shows no cross-reaction with pathogens such as UU, CP, TB, EBV and influenza virus.

List of references

1. Rothstein Tanner E,Cunningham Scott A,Rieke Rachelle A,Mainella Jill M,Mutchler Melissa M,Patel Robin. Macrolide Resistance in *Mycoplasma pneumoniae*, Midwestern United States, 2014 to 2021.[J]. Antimicrobial agents and chemotherapy,2022.

2. Wan Qianyi,Li Wei,Zhao Mingyuan,Wang Haixia,Li Yang,Shi Chao,Ma Cuiping. Performance Analysis of Novel Nucleic Acid Detection Kit for *Mycoplasma pneumoniae*.[J]. Clinical pediatrics,2022,61(4).

3. Heping Wang,Qian Zhou,Wenkui Dai,Xin Feng,Zhiwei Lu,Zhenyu Yang,Yanhong Liu,Gan Xie,Yonghong Yang,Yonghong Yang,Yonghong Yang,Kunling Shen,Kunling Shen,Yinhu Li,Shuai Cheng Li,Ximing Xu,Yongshun Shen,Dongfang Li,Dongfang Li,Yuejie Zheng. Lung Microbiota and Pulmonary Inflammatory Cytokines Expression Vary in Children With Tracheomalacia and Adenoviral or *Mycoplasma pneumoniae* Pneumonia[J]. Frontiers in Pediatrics,2019,7.

4. Tsai Ti An,Tsai Chang Ku,Kuo Kuang Che,Yu Hong Ren. Rational stepwise approach for *Mycoplasma pneumoniae* pneumonia in children[J]. Journal of Microbiology, Immunology and Infection,2020,54(prepublish).

5. Wan Ruijie,Jia Minyi,Dou Haiwei,Tu Peng,Shi Dawei,Yuan Qing,Xin Deli. Mechanism of Infantile Feire Kechuan Oral Solution against *Mycoplasma pneumoniae* infection of A549 cells.[J]. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie,2021,145.

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
Σ	Contains sufficient for <n> tests		Temperature Limit
	Caution		Consult Instructions for Use
21 PAP	PAP21: Not corrugated cardboard		Do not re-use
PCR MIX	PCR Mix	ENZYME MIX	Enzyme Mix
CONTROL -	Negative Control	CONTROL +	Positive Control
REFERENCE + A	Positive Reference A	REFERENCE + C	Positive Reference C
REFERENCE + B	Positive Reference B	REFERENCE + D	Positive Reference D
LYSIS BUFFER	Lysis Buffer	INTER CONTROL	Internal Control
CONCENTRATE	Concentrate	VERSION	Version
PRE	Prepackaging		Keep away from light
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.
UDI	Unique device identifier		

Contact information

Sansure Biotech Inc.

Add.: No. 680, Lusong Road, Yuelu District, 410205 Changsha, Hunan Province, PEOPLE'S REPUBLIC OF CHINA

Tel.: +86-731-88883176

Fax: +86-731-88884876

Web: www.sansureglobal.com

EC REP **Obelis s.a.**

Bd. Général Wahis 53, 1030 Brussels, BELGIUM

Tel: + (32) 2.732.59.54

Fax: + (32) 2.732.60.03

E-Mail: mail@obelis.net

CE IVD