Mycobacterium Tuberculosis and Rifampicin Resistance Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Catologue Number

S3363E-12-P

Product Name

Mycobacterium Tuberculosis and Rifampicin Resistance Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)

Packaging Specification

Pre-packaged 12 tests/kit

Intended use

The Mycobacterium Tuberculosis and Rifampicin Resistance Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is a real-time polymerase chain reaction test kit intended for the qualitative detection of the nucleic acid of mycobacterium tuberculosis and rifampicin resistance mutations in human sputum samples. The test results can be used to assist in the diagnosis of TB patients and patients with an increased risk of RFP drug-resistant TB, providing a molecular diagnosis basis for infected patients.

The highly conservative multi copy insertion sequences IS6110 and IS1081 of mycobacterium tuberculosis are detected to diagnose the infection of mycobacterium tuberculosis; the mutations of a total of 27 amino acid codon regions (81bp, determining regions of rifampicin resistance) that from the amino acid 507th to 533rd in the rpoB gene of mycobacterium tuberculosis complex are detected to perform resistance screening. The test results of this diagnostic kit are only for clinical reference and should not be used as the only standard for clinical diagnosis. It is suggested to make a comprehensive analysis of patients' conditions in combination with clinical manifestations and other laboratory tests.

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

Summary

Mycobacterium tuberculosis (*M. tuberculosis*) is the pathogen causing tuberculosis, which can invade all organs of the whole body, and pulmonary tuberculosis caused by pulmonary involvement is the most common. Early diagnosis and treatment are crucial measures to effectively control the spread of tuberculosis. Due to the abuse of antibiotics or the insufficient course of drugs of patients, the sensitivity of patients to drugs weakens or even disappears, resulting in the decreasing or ineffective effect of drugs on pulmonary tuberculosis. According to the types of anti-tuberculosis drugs, drug-resistant tuberculosis can be divided into monoresistance pulmonary tuberculosis, polyresistance pulmonary tuberculosis, multidrug resistance pulmonary tuberculosis and extensively drug-resistant pulmonary tuberculosis. Rifampicin is one of the first-line drugs for the treatment of pulmonary tuberculosis. The resistance of mycobacterium tuberculosis to rifampicin is related to the mutation of RNA Multiple Polymerase β Subunit (rpoB gene), which enables rifampicin not to bind to mycobacterium tuberculosis, resulting in drug resistance.

Test Principle

This diagnostic kit can rapidly lyse samples and release mycobacterium tuberculosis DNA with a nucleic acid release agent. Using two pairs of specific primers and two specific fluorescent probes designed for the nucleic acid conservative regions of mycobacterium tuberculosis, with PCR reaction solution and other components, applying real-time fluorescent quantitative PCR detection technology on a fluorescence quantitative PCR instrument, the rapid detection of mycobacterium tuberculosis DNA can be realized by the change of fluorescence signal. At the same time, using a pair of specific primers and five specific fluorescent probes designed in the RRDR region of rpoB gene, with PCR reaction solution and other components, applying real-time fluorescent quantitative PCR detection technology on a fluorescence quantitative PCR instrument, whether the probes have amplification curve can be detected by the change of real-time monitoring fluorescence signal, further determining the mutation information of this sequence. If the target sequence matched the probes perfectly, the probes hybridized with this sequence and generate the amplification curve of the corresponding fluorescent channel; if the probes did not match the sequence perfectly (e.g. there are point mutations, insertions or deletions in this sequence, the probes cannot hybridize with this sequence and cannot generate the amplification curve of the corresponding fluorescent channel.

Components of the Diagnostic Kit

The diagnostic kit contains the following components:

No.	Reagent Name	Spec. & Qty.	Main Ingredients
1	Sample Release Reagent	40μL/tube × 24tubes	KCl, Sodium dodecyl sulfate
2	TB and RFP-PCR Mix A	38μL/tube × 12tubes	Primers, Probes, $MgCl_2$, dNTPs and PCR buffer
3	TB and RFP-PCR Mix B	38μL/tube × 12tubes	Primers, Probes, $MgCl_2$, dNTPs and PCR buffer
4	TB and RFP-Enzyme Mix	2μL/tube × 24tubes	Taq enzyme
5	TB and RFP-Negative Control	500μL/tube × 1tube	Normal saline
6	TB and RFP-Positive Control	500μL/tube × 1tube	Plasmids containing tuberculosis IS6110,IS1081, rpoB gene and Internal Control segments

Note:

1. Do not mix or exchange components from different product lots.
2. Materials required but not provided: 2.0 mL DNase-free centrifuge tubes, various types of pipettes and pipette tips (10 μL, 200 μL and 1000 μL tips with filters); a micro-centrifuge; a vortex mixer; and a ultrasonic crusher.
3. Reagent required but not provided: normal saline and Sample Diluent (Reference Number: Y1001E) manufactured by Sansure Biotech Inc.

Storage and Stability

1.The diagnostic kit should be stored in the original kit at the temperature from -25℃ to -15℃ and protected from light. The kit is valid for 12 months.

2.Please refer to the manufacturing date and expiry date on the outer package.

3.Unopened reagents are valid and stable until the expiry date.

4.Once the reagents are opened, the maximum number of freeze/thaw cycles should not exceed three.

5.The reagents keep valid and stable before the expiry date on the outer package when transporting for up to 15 days in a sealed foam box containing coolant with the temperature lower than 20°C.

Compatible Instruments

This product is applicable for Portable Molecular Workstation (S-Q36A).

Specimen Requirements

1. Applicable type of samples: Sputum
2. Specimens collection:

The first sputum in the morning is regarded as the best sample. Rinse the mouth with clean water first, and cough up deep sputum forcefully, keep the sample in the sterile sample storage tube, seal the tube, and submit the tube for examination.

3. Storage and delivery of specimens

The above collected samples can be used for test immediately, or stored in an environment from 2℃ to 8℃ (within 24 hours). Samples that cannot be detected within 24 hours should be stored at -70 °C or below for long-term storage (in the absence of -70 °C or below storage conditions, the samples can be stored at -25 to -15℃ for 12 months).The samples should be transported in a sealed foam box with ice.

Test Method

1. Follow the following steps:

1.1 Reagent preparation (conducted in the reagent preparation area)

1.1.1 Take out each component from the diagnostic kit and place them at room temperature. Allow the reagents to equilibrate at room temperature, then shake each of them by hand respectively until the liquid into the bottom of tube for later use.

1.2 Sample treatment and sample application (conducted in the reagent treatment area)

1.2.1 Sample treatment

Add 2-3 times the volume of sample diluent (Reference Number: Y1001E) manufactured by Sansure Biotech Inc. to samples, shake to mix it and then let stand for liquefaction for 5-10 minutes. Take 1.5 mL of the liquefied sample into a 2.0 mL centrifuge tube (do not take distinct solid impurities), then conduct ultrasonic treatment for 15 seconds, stop for 5 seconds, and repeat the process for 3 times, which takes a total of one minute. The treated sample is used as the sample to be tested.

1.2.2 Sample application (the negative control can be treated synchronously with the sample to be tested)

1.2.2.1 Put the blue single tube containing Sample Release Reagent into the well B; Put the brown single tube containing TB and RFP-PCR Mix A or TB and RFP-PCR Mix B into the well C; Put the purple single tube containing TB and RFP-Enzyme Mix into the well D. (There is no need to remove the sealing plug of the brown single tube and purple single tube, which are put into to the carrier set).

1.2.2.2 Remove the sealing plug of the blue single tube containing Sample Release Reagent, and discard the sealing plug in the medical trash can, and then add 40 μL treated samples or TB and RFP-Positive Control or TB and RFP-Negative Control into Sample Release Reagent. (In order to avoid bubbles during operation, it is recommended to pipette deeply and release slowly).

1.3 PCR amplification (please refer to the operating instructions of each instrument for setting)

1.3.1 Click the "" and "" buttons on the instrument display screen to open the door of the instrument, and put the prepared consumables into the designated position of the instrument.

1.3.2 Click "New" on the instrument display screen to enter a new experimental task setting interface.

1.3.3 Select required experimental projects in the drop-down menu of "Experimental projects", enter the corresponding task names in the task name column, and enter and select other items to be entered or selected.

1.3.4 Click "Submit" to submit experimental tasks, click "OK" to run the instrument and start the experimental tasks in turn.

2. Result analysis (please refer to the operating instructions of each instrument for setting)

Results will be automatically saved after reaction, and adjust the Start value, End value and Threshold value of Baseline according to the analyzed images (the values can be adjusted according to the actual situation. The Start value can be set within 3-15 and the End value can be set within 5-20. Adjust the amplification curve of the negative control to be straight or lower than the threshold line), click "Analyze" to analyze and make all parameters meet the requirements in "**3. Quality control**" below, and then record the Ct values in the Plate window.

3. Quality control

3.1 TB and RFP-Negative Control: There are no Ct values or Ct values are >35 in FAM, HEX, ROX and CY5 of both MIX A and MIX B..

3.2 TB and RFP-Positive Control: Ct values are ≤35 in FAM, HEX, ROX and CY5 of both MIX A and MIX B.

3.3 The above requirements should be met at the same time in the same experiment, otherwise, the experiment is invalid and needs to be conducted again.

Reference Range

The Ct reference value for the test of target genes by this diagnostic kit was determined to be 35 by the study of reference values, and that for the Internal Control was 35.

Explanation of Detection Result

1.Explanation of the detection result

PCR MIX A				PCR MIX B				Results
HEX	FAM	CY5	ROX	FAM	HEX	ROX	CY5	
Ct≤35	Any channel Ct≤35		All channels Ct≤35					MTB DETECTED, RIF Resistance NOT DETECTED
			Any channel Ct＞35 or No Ct					MTB DETECTED, RIF Resistance DETECTED
	All channels Ct＞35 or No Ct		Not considered					MTB NOT DETECTED
Ct＞35 or No Ct	Not considered							Invalid

2.The target genes corresponding to the fluorescent channels

Chanels	FAM	HEX	ROX	CY5
Target gene of MIX A	IS6110	IC	rpoB-A	IS1081
Target gene of MIX B	rpoB-E	rpoB-C	rpoB-B	rpoB-D

Limitations of Detection Method

1. The test results of this diagnostic kit are only for clinical reference. The clinical diagnosis and treatment for patients should be considered in combination with their symptoms / signs, medical history, other laboratory tests and treatment response.
2. Possibility analysis of false-negative results

2.1 Unreasonable sample collection, treatment, transport and low sample concentration may result in false-negative results.

2.2 The variation of target sequences to be tested or sequence changes caused by other reasons may result in false-negative results.

2.3 Unreasonable reagent storage may result in false-negative results.

2.4 Other unverified interference factors or unverified PCR inhibitory factors may result in false-negative results.

3. Cross contamination occurring during sample treatment may result in false-positive results.
4. Clinical laboratories should be equipped with equipment and operators strictly according to the requirements of *The Work Specification for Clinical Gene Amplification Laboratories*, and all operations should be conducted strictly according to the requirements of the instructions.

Product performance index

1. Accuracy

The positive reference products of enterprises were tested, and the results were all positive.

2. Specificity

This diagnostic kit has no cross reaction with *mycoboterium kansasii*, *mycobacterium marinum*, *mycobacterium terrae*, *mycobacterium triviale*, *ulcer mycobacterium*, *gordon mycobacterium*, *mycobacterium bufo*, *mycobacterium avium*, *mycobacterium scrofulaceum*, *mycobacterium szulgai*, *mycobacterium chelonei*, *mycobacterium abscessus*, *mycobacterium smegmatis, accidental mycobacterium, mycobacterium gastricum, intracellular mycobacterium, mycobacterium phlei, streptococcus pneumoniae, haemophilus influenzae, escherichia coli, staphylococcus epidermidis, cryptococcus, staphylococcus aureus, nocardia, pseudomonas aeruginosa, candida albicans*, human DNA and other samples. The negative reference products of enterprises were tested, and the results were all negative.

3. Precision

The coefficients of variation (CV, %) of intra-batch / inter-batch and intra-day / inter-day are 5%.

4. Limit of detection

The minimum limit of detection of this diagnostic kit for mycobacterium tuberculosis is 1×10^3 bacteria/mL, and the minimum limit of detection for rifampicin resistance is 1×10^4 bacteria/mL.

5. The reagent can detect heterogeneous drug-resistant strains with 35% or more mutant strains.

6. Interference immunity

Potential interfering substances in samples: isoniazid (6 μg/mL), rifampicin (25 μg/mL), levofloxacin (17 μg/mL), oxymetazoline (0.5 mg/mL), mupirocin (0.8 mg/L), ethambutol (7 μg/mL), pyrazinamide (50 μg/mL), zanamivir (140 ng/mL), dexamethasone (5 mg/mL), hemoglobin (20 mg/mL), blood (5%) and mucoprotein (10 μg/mL) have no distinct interference to the test results of this diagnostic kit.

Precautions

1. This product is for in vitro diagnosis only; please read the instructions carefully before use.
2. Before experiments, please be familiar with and master the operation methods and precautions of various equipment to be used, and conduct quality control for each experiment.
3. Laboratory management should strictly follow the management specifications of PCR gene amplification laboratories, experimenter must receive professional training, experiments should be conducted strictly in different areas, all consumables are disposable and should be sterilized, specialized instruments and equipment should be used in each stage of the experimental operation, and laboratory supplies of all areas and stages should not be used interchangeably.
4. All test samples should be deemed to be infectious; experimenters should wear working clothes and disposable gloves during experiments and often alter the gloves to avoid cross contamination between samples; and sample operation and waste disposal should meet the requirements of relevant laws and regulations of the Ministry of Health: General Guidelines for Biosafety of Microbiological Biomedical Laboratories and Medical Waste Management Regulations.
5. If the diagnostic kit is transported in a foam box with refrigerant for 15 days and the temperature is not higher than 20℃, the validity term of this product will not be affected.

Bibliography

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[3] Jagielski T, Minias A, van Ingen J, et al. Methodological and Clinical Aspects of the Molecular Epidemiology of Mycobacterium tuberculosis and Other Mycobacteria. Clin Microbiol Rev. 2016 Apr;29(2):239-90.

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Symbols

Symbols	Meanings	Symbols	Meanings
IVD	*In vitro* Diagnostic Medical Device		Date of Manufacture
	Use by date		Consult Instructions for Use
	Temperature Limitation		Manufacturer
LOT	Batch code	REF	Catalogue Number
Σ	Contains sufficient for <n> tests		Cautions

Basic information

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IVD

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