Diagnostic Kit for Mycobacterium Tuberculosis(MTB) complex DNA and Rifampicin resistance Mycobacterium tuberculosis(PCR-Fluorescence)

REF 106-0056-01

Product Name
Diagnostic Kit for Mycobacterium Tuberculosis(MTB) complex DNA and Rifampicin resistance Mycobacterium tuberculosis(PCR-Fluorescence)

Specification
12 reactions/kit

Intended Use
This kit is used for the in vitro qualitative detection of Mycobacterium tuberculosis complex nucleic acids in sputum samples and rifampicin resistance in Mycobacterium tuberculosis complex-positive sputum samples from tuberculosis patients. This product cannot identify specific mutations type. Utilize real-time multiplex fluorescence PCR technology with Automated Fully Enclosed qPCR Instrument for in vitro extraction and amplification automatically. It is used for the auxiliary diagnosis of Mycobacterium tuberculosis and rifampicin-resistant.
Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis. Tuberculosis can occur in any part of the body, most commonly in the lungs. When tuberculosis patients cough, sneeze, or talk loudly, droplets containing tuberculosis bacteria will be spread into the air. Healthy people may become infected by inhaling the droplets emitted by infectious tuberculosis patients. Globally, TB is one of the top ten causes of death and the leading cause of death from infectious diseases (ranking ahead of HIV in mortality since 2007). As the contagious nature of tuberculosis, rapid and accurate diagnosis is an important factor in tuberculosis prevention, control and treatment.

Test Principle
This kit combines the magnetic bead extraction method and real-time multiplex fluorescence PCR technology to design primers and probes for the IS6110 gene fragment and the 81bp rifampicin resistance core region (RRDR) of the RpoB gene, respectively to detect the presence of the Mycobacterium tuberculosis complex (AMCA labeling), and whether the Mycobacterium tuberculosis has undergone rifampicin-resistant mutations (FAM, HEX, CY5.5 CY5.5, ROX and CY5 markers), which in turn can be used to assist in the diagnosis of tuberculosis or rifampicin resistance. The kit is designed with an internal control, which is used to monitor the extraction and amplification of the reagents. The extraction and amplification are automated in a closed circuit, avoiding contamination by humans or the environment, increasing the accuracy of the test, and avoiding aerosol spillage contamination.

Components

IGS TB/RIF Lite iCassette Composition	Composition Name	Quantity
IGS TB/RIF Lite iCassette	Built-in nucleic acid extraction components, internal control and PCR buffer, enzyme, probe	12 pcs
Pretreatment solution	NaOH	2 x 28mL/bottle
Enhancer	/	1 tube (1mL)
TB positive control	TB plasmid	1 tube (200μL)
TB negative control	Sterilizing purified water	1 tube (200μL)

Note: Components in different lot numbers are not interchangeable.

Storage conditions and Shelf Life
The kit should be stored at 2-8℃ and the shelf life of this kit is 9 months. The transportation temperature range of the kit should be kept at 2-8℃.
Do not open the iCassette lid before adding the sample. If you open the iCassette lid, it must be used within 30 minutes.

Applicable Instrument
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.
- Ultrosonic machine: Galaxy USL.
- 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.
- Saline.

Sample Requirements
1. Sample types: Sputum
2. Sample collection
Sputum collection：Use spit cup to take 1-2 mL of sputum coughed up from the deep lungs of the patients and seal it immediately. The samples can be used for testing immediately or stored at -20℃ or below for future testing. The storage period is 6 months.
3. Principles of Biosafety Protection
All operations should comply with relevant local laws and regulations.

Test Method and Operation
1. Prepare the IGS TB/RIF Lite iCassette
1.1 Process the samples:
A. Pre-process the sputum: add 2 times the sample volume of pretreatment liquid, then add the appropriate amount of enhancer in the proportion indicated in Table 1, shake vigorously for 1 minute, and leave for 15 minutes at room temperature to liquify, shake for 10 seconds every 5 minutes. The sample should be fully liquified and free of tiny lumps of unliquified sputum.

Table 1 Proportion of Added Enhancer

Sputum specimen	Pretreatment liquid	Total volume	Enhancer
1mL	2 mL	3mL	30μL
2mL	4 mL	6mL	60μL

B. Ultrasonic Crushing:
Open IGS TB/RIF Lite iCassette package,observe whether the lyophilization in the tube are intact. Pipette 1 mL of the liquefied sample and slowly add it to the IGS TB/RIF Lite iCassette sample compartment as shown in Figure1, 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)
C. Place the ultrasonicated iCassette into the instrument.

1.2 Process negative and positive controls
A. Pipette 400 μL of saline and 200 μL of TB positive control or negative control into IGS TB/RIF Lite iCassette sample compartment as shown in Figure1, close the lid tightly, put it into the ultrasonic machine, and sonicate for 20 seconds.
B.Place the ultrasonicated iCassette into the instrument.

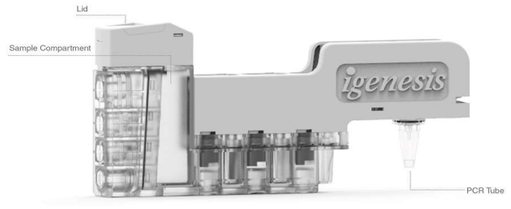

Figure 1 IGS TB/RIF Lite iCassette (Lateral View)

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: When the sample test result is negative, the internal control TAMRA channel must be positive; when the sample test result is positive for Mycobacterium tuberculosis, the internal control TAMRA can be positive or negative.
Probe quality control: if the fluorescence signal measurement of the probe displays "probe check fail", it indicates that there is a problem with the probe in the iCassette and the iCassette is unavailable; If the PCR step is performed after the iCassette self-test, it indicates that the iCassette probe is qualified.
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.
The results of positive control are positive in all channels, TAMRA can be positive or negative.
The results of negative control are negative, only TAMRA can be positive.

Result Judgment
Positive judgment for Mycobacterium tuberculosis complex：When the Ct value of AMCA channel is less than or equal to 40, the test result is positive.
Judgment of rifampicin resistance Mycobacterium tuberculosis： When the Ct value of the AMCA channel is less than or equal to 40 and the Ct value of any channel of FAM, HEX, ROX, CY5 or CY5.5 is 0 or all greater than 40, indicates the test result is positive .
Negative judgment for Mycobacterium tuberculosis complex: When the Ct value of AMCA, FAM, HEX, ROX, CY5, CY5.5 is 0 or all greater than 40, and TAMRA channel is less than or equal to 40, indicates the test result is negative.

Reference Interval
Through the analysis of clinical sample test results, the ROC curve method is used to finally determine the Ct reference value of this kit is 40.

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

AMCA Channel	FAM Channel	HEX Channel	ROX Channel	CY5 Channel	CY5.5 Channel	TAMRA Channel	Result
√	×	√	√	√	√	×/√	MTB DETECTED; Rif Resistance DETECTED(Figure 2)
√	√	×	√	√	√	×/√	MTB DETECTED; Rif Resistance DETECTED
√	√	√	×	√	√	×/√	MTB DETECTED; Rif Resistance DETECTED
√	√	√	√	×	√	×/√	MTB DETECTED; Rif Resistance DETECTED
√	√	√	√	√	×	×/√	MTB DETECTED; Rif Resistance DETECTED
√	√	√	√	√	√	×/√	MTB DETECTED; Rif Resistance NOT DETECTED(Figure 3)
×	×	×	×	×	×	√	MTB NOT DETECTED;
√	√	√	√	√	√	×/√	Positive control
×	×	×	×	×	×	√	Negative control

Note:“√”indicates that the result "has an obvious logarithmic amplification curve, and ct≤40"; “×”indicates the result "no logarithmic amplification curve and ct＞40"; “×/√”indicates the result "with obvious amplification curve or no amplification".

Figure 2 MTB DETECTED; Rif Resistance DETECTED

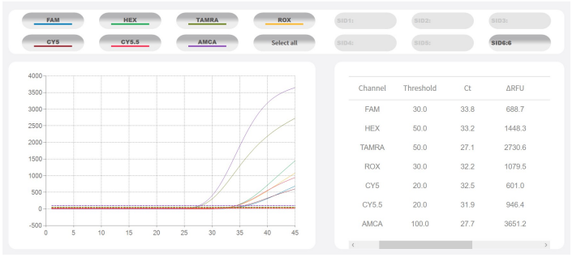

Figure 3 MTB DETECTED; Rif Resistance NOT DETECTED

Limitations of Test Method

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.The kit is only suitable for auxiliary detection of Mycobacterium tuberculosis infection and rifampicin resistance mutations.

3.Unreasonable sample collection, transportation and processing, as well as improper experimental operation and experimental environment may lead to false negative or false positive results.

4. The variation of the target sequence of enterovirus to be tested or the sequence change caused by other reasons may lead to false negative results.

Product performance Index

1.Analysis Sensitivity

1.1 Take the minimum detection quantity reference material of "China National Reference Material for Mycobacterium Tuberculosis PCR Detection Kit" for detection and the sensitivity of detecting single cell of Mycobacterium tuberculosis is 100 CFU/mL or 100copies/mL.

1.2 Take the lowest detectable quantity reference product of "Reagent for rifampicin resistance gene detection of Mycobacterium tuberculosis of China National Reference Material" detection and the sensitivity of detecting single-cell drug-resistant Mycobacterium tuberculosis is 100CFU/mL or 100 copies/mL.

2.Analysis Accuracy

2.1 Take the positive reference materials P1-P15 from the "National Reference Materials for Mycobacterium tuberculosis PCR Detection Kit", and the test results are all positive for Mycobacterium tuberculosis complex.

2.2 Take the positive reference materials R1-IR-1 to R1-IR-17 from the "National Reference Materials for Rifampicin Resistance Gene Detection Reagents of Mycobacterium tuberculosis", and the test results are all corresponding drug resistance.

See Table 2 for specific mutation points：

Table 2 ： "National Reference Materials for Mycobacterium tuberculosis Rifampicin Resistance Gene Detection Reagents" Positive Reference Material Numbers and Corresponding Mutation Points

Number	Mutation Points	Test results
R1-IR-1	Rpob531	Postive
R1-IR-2	Rpob516	Postive
R1-IR-3	Rpob531	Postive
R1-IR-4	Rpob526	Postive
R1-IR-5	Rpob526	Postive
R1-IR-6	Rpob526	Postive
R1-IR-7	Rpob531	Postive
R1-IR-8	Rpob516	Postive
R1-IR-9	Rpob531	Postive
R1-IR-10	Rpob526	Postive
R1-IR-11	Rpob522	Postive
R1-IR-12	Rpob510;511;512Site deletion	Postive
R1-IR-13	Rpob526	Postive
R1-IR-14	Rpob531	Postive
R1-IR-15	Rpob513	Postive
R1-IR-16	Rpob533	Postive
R1-IR-17	Rpob517;518Site deletion	Postive

3.Analysis Specificity

Take the negative reference product N1-N15 of "China National Reference Material for Mycobacterium Tuberculosis PCR Detection Kit" at a concentration of 1×10^4 CFU/mL for testing.The test results are all negative for Mycobacterium tuberculosis. See the specific strain name Table 3：

Table 3: "National Reference Materials for Mycobacterium tuberculosis PCR Detection Kits" negative reference product number and corresponding strain name

Number	Strain name	Test results
N1	Mycobacterium avium	Negative
N2	Mycobacterium terrestrial	Negative
N3	Mycobacterium stutzeri	Negative
N4	Mycobacterium kansas	Negative
N5	Mycobacterium asiaticum	Negative
N6	Mycobacterium scrofula	Negative
N7	Mycobacterium gordonii	Negative
N8	Mycobacterium pyogenes	Negative
N9	Mycobacterium fortuitum	Negative
N10	Mycobacterium phlei	Negative
N11	Mycobacterium brasinoca	Negative
N12	Corynebacterium pekinensis	Negative
N13	Pneumococcus	Negative
N14	Legionella pneumophila	Negative
N15	Bordetella pertussis	Negative

4.Precision

4.1Take the precision reference J1-J10 of "China National Reference Material for Mycobacterium Tuberculosis PCR Detection Kit" at a concentration of 1×10^2 CFU/mL for testing, and the test results are all positive and the value of CV should be less than or equal to 10%.

4.2 Take the precision reference product J of "Reagents for the detection of rifampicin resistance genes of Mycobacterium tuberculosis", and dilute it to a concentration of 1×10^3 CFU/mL for testing. The test results are all positive and the value of CV should be less than or equal to 10%.

5. Cross-reactivity

This kit detects non-tuberculous mycobacteria (including Mycobacterium kansasii, Mycobacterium marinum, Mycobacterium terrestrialis, Mycobacterium minora, Mycobacterium ulcerans, Mycobacterium gordonii, Mycobacterium toadstool, Mycobacterium avium, Mycobacterium, Mycobacterium scrofulaceum, Mycobacterium thuka, Mycobacterium chelonae, Mycobacterium abscessus, Mycobacterium smegmatis, Mycobacterium fortuitum, Mycobacterium gastricus, Mycobacterium intracellulare, Mycobacterium phylla bacilli) and other pathogens, the results were negative and there was no cross-reaction.

This kit detects the following pathogens, including: Streptococcus pneumoniae, Haemophilus influenzae, Escherichia coli, Staphylococcus epidermidis, Cryptococcus, Staphylococcus aureus, Nocardia, Pseudomonas aeruginosa, Candida albicans, and human parainfluenza virus (types 1, 2 and 3) and human influenza viruses (types A and B), the results were all negative and there was no cross-reaction.

Interfering Substances

This kit detects the following exogenous drugs: isoniazid (12μg/mL), rifampicin (12μg/mL), amoxicillin (10μg/mL), oxymetazoline hydrochloride (0.5mg/mL), moxazolin Pilocin (20μg/mL), ethambutol hydrochloride (7μg/mL), pyrazinamide (12μg/mL), zanamivir (160ng/mL), dexamethasone (0.5mg/mL), kanamycin (35μg/mL) and streptomycin (50μg/mL) were negative and there was no cross-reaction.

This kit detects the following endogenous substances: blood (5%), mucin (2mg/mL), human genome (2.5μg/mL) and will not affect the test results.

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. In order to prevent contamination of the sample, the experimenter should take good care of it and wear disposable gloves and masks.

5. Each iCassette is single-used, 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; The operation and processing of sample must meet the requirements of local relevant laws and regulations:.

References

1. Resistance profifiles and rpoB gene mutations of Mycobacterium tuberculosis isolates in Taiwan ， Yun-Ho Lin a , Journal of Microbiology, Immunology and Infection (2012), 1e5

2. Drug resistance of Mycobacterium tuberculosis to rifampin and RRDR mutation in the India D gene， Diagnostics Theory and Practice Volume 1,17,(2018)

3. Mutations inside rifampicin-resistance determining region of rpoB gene associated with rifampicin-resistance in Mycobacterium tuberculosis， Myo T. Zaw,Journal of Infection and Public Health 11 (2018) 605–610

Instruction Version

Version: A/2

Date of Issue: May,2021

Last revised: April, 2024

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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