DiAGSure Mycobacterium tuberculosis (MTB) Detection Kitit

Description:

Tuberculosis (TB) is caused by the acid-fast bacterium Mycobacterium tuberculosis. Although Mycobacterium tuberculosis most commonly infects the lungs (pulmonary tuberculosis), several extrapulmonary cases of TB are also reported where the bacteria spreads to the central nervous system, lymphatics, urogenital system, bones and joints and is more frequent in immuno-compromised individuals. The disease is transmitted through aerosolized droplets from infected individuals. The symptoms include fever, fatigue, chills, night sweats, appetite loss and weight loss. However, asymptomatic cases of latent tuberculosis are often reported. Tuberculosis has become a major public health concern worldwide. According to WHO statistics, India contributes to the highest TB burden in the world with about 2.2 million cases each year. Among the different means of TB diagnosis, polymerase chain reaction (PCR) has been proven to be extremely useful and a sensitive diagnostic tool.

DISCLAIMER: The DiAGSure MTB Detection Kit has been designed for in-vitro use only.

Intended Use:

This kit detects a conserved 369-bp region in the RD9 gene specific for M. tuberculosis and is absent in all other species of

mycobacteria. This kit also contains an internal control which is set-up in a separate tube and amplifies a 221-bp region from human DNA. This internal control has been included to ensure proper DNA extraction and PCR reaction in the absence of amplification in the target sequence.

Principle:

The DiAGSure Chikungunya Detection Kit involves semi-quantitative RT-PCR based detection of a conserved CHIKV 212-bp sequence using gene-specific primers. PCR-based detection is emerging as a highly sensitive diagnostic tool for the detection of pathogen in a wide array of clinical samples. Reverse transcriptase converts the viral RNA to cDNA which serves as a template for PCR. A basic PCR reaction involves three basic steps:

i. Denaturation, where separation of the two DNA strands occur
ii. Annealing, where the primers are allowed to anneal to their cognate templates
iii. Extension, where the actual amplification occurs that is repeated between 25 and 40 cycles in each assay. The PCR primers have been designed to ensure high specificity and sensitivity.

Features:

- ✓ Fast and simple
- ✓ Rapid detection of TB pathogen in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the M. tuberculosis pathogen
- ✓ Reproducibility of results

Storage and Shelf life:

The provided kit has a shelf-life of 6 months when stored at -20°C. Repeated thawing and freezing of PCR reagents may reduce the sensitivity and therefore should be avoided. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. The degradation of sample DNA specimens may also compromise with the sensitivity of the assay. Usage of the kit after the expiry date stated on pack is not recommended.

Kit contents:

Table for kit of 100 tests:

Part 1 of 4 (Storage: Room temperature)

GSure Viral RNA Mini kit (Cat. No. XXXXX) – 20preps

Part 2 of 4 (Storage: Room temperature)

Kit Contents	Catalog No.	Volume for 100 tests
Agarose	G4652	100g
GPure TAE Buffer, 40X	GS1006	220mL
0.2mL PCR tubes		350 no.s
1.5mL microcentrifuge tubes		100 no.s
Ethidium Bromide Solution (10mg/ml)	GCR-28	500µL

N.B.: On receipt, store the Ethidium bromide solution at 4°C.

Part 4 of 4 (Storage: -20°C)

Kit Contents	Catalog No.	Volume for 100 tests
MTB Primer mix	-	330µl
DiAGPol PCR Master Mix	G7117	1.5mL X 2
DiAGSure DNA ladder		500µL
Positive control primer mix		330µl
6X Gel loading dye	GCR-19	1.75mL
Nuclease free water	GCR-30	1.5mL X 3

Sample Material Preparation:

The DiAGSure MTB Detection Kit detects the presence of M. tuberculosis in human blood and sputum samples. Isolate total DNA from blood or sputum (which includes bacterial DNA in case of infected samples) using GSure Blood DNA isolation kit or GSure Sputum DNA isolation kit provided with this kit. Use a specified amount (see below) of this DNA to amplify the 369-bp region of the RD9 gene.

Basic workflow:

Isolate total DNA from blood or sputum

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Check the DNA purity by running it in a 1% agarose gel and quantitate it in a spectrophotometer or a nano-drop

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Set up the PCR reaction

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Run the PCR products in a 2% agarose gel

PCR Protocol:

Set up a 25µL PCR reaction by adding the following constituents in a PCR tube:

Nuclease-free water –	6.5µL
2X G9 Taq PCR Master Mix –	12.5µL
MTB Primer mix –	1µL
Template DNA –	5µL

PCR conditions:

Stage	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	30 secs	35
Annealing	60	30 secs	
Extension	72	30 secs	
Final extension	72	5 mins	1
Final hold	4	∞	1

Agarose gel electrophoresis and gel visualization:

1. Cast a 2% agarose gel as follows. Weigh out 1g of agarose and add it to 50mL of 1X TAE buffer taken in a conical flask and heat it until the agarose has dissolved completely. Cool the flask slightly and add 2µL of

10mg/mL Ethidium bromide solution and pour immediately into the gel casting tray. After gel solidification, place the gel in 1X TAE buffer for 15mins for equilibration.

2. Add 1µL of 6X DNA loading dye to the PCR product and load it into appropriate wells of the gel placed in the gel running apparatus containing fresh 1X TAE buffer. Visualize the gel in a uv-transilluminator or a gel documentation instrument.

Results Interpretation:

The presence of a band of 212-bp appearing in between 200bp and 300bp with respect to the standard marker indicates the presence of Chikungunya virus in the clinical sample. The absence of the 212-bp band n the test sample indicates the absence of Chikungunya infection (See Fig 1).

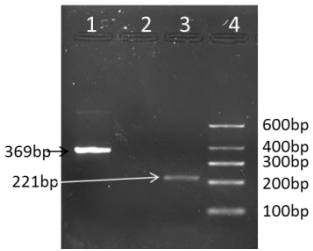

Fig 1. **Representative gel image showing amplification of the MTB gene. Lane**
1: Positive amplification at 369-bp; Lane
2: Negative control; Lane
3: 221-bp Internal control; Lane
4: DiAGSure DNA ladder

Sensitivity:

The DiAGSure MTB Detection Kit can detect ≥ 2100 copies of the bacterium.

Quality Control:

All reagents in the DiAGSure MTB Detection Kit are free from endonuclease and exonuclease activities and the kit has been functionally tested for amplification.

Troubleshooting Guide:

1. *No amplification in internal control*
- Ensure that DNA has been properly isolated.
- Use freshly isolated DNA for amplification.
- Verify that all reagents are added to the PCR reaction in indicated amounts and proper PCR conditions have been maintained.
- It is advisable to store the reagents in aliquots for multiple uses.

2. *Amplification in negative control*
- Indicates that reagents have been contaminated. Repeat the reaction using a fresh aliquot.

3. *Variability among replicates*
- This can be due to manual pipetting error. In case of multiple replicates, prepare a master mix and aliquot it into replicate tubes.

4. *Clear gel with no bands*
   - Ensure that EtBr has been added to the gel.
   - Ensure mild cooling of the gel post dissolution of the agarose prior to EtBr addition.

Safety information:

The DiAGSure MTB Detection Kit is for laboratory use only. Use proper safety measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

Technical assistance:

Satisfaction of the customers is our utmost priority. For any kind of technical assistance, always feel free to reach out to us at tech.support@gccbiotech.co.in