CE
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HELINI Mycobacterium Tuberculosis [MTB] & Multidrug resistance [MDR] Real-time PCR Kit

Instruction manual

Cat.No:8224 – 25/50/100 tests

Compatible with: Agilent, Bio-Rad, Roche Lightcycler, Cobas-480, Applied Bio systems [ABI], Rotor-gene, Cepheid and Spartan Real time PCR machine.

www.helini.in

Kit components

No. of reactions	25
Probe PCR Master Mix -10µl/reaction	4 x 250µl
MTB Primer Probe Mix [MTB PP Mix] -2.5µl/reaction]	65µl
Internal control Primer Probe Mix [IC PP Mix – 2.5µl/reaction]	65µl
Internal control template [IC template] -5µl/reaction	125µl
rpoB Primer & Probe Mix [rpoB PP Mix] -5µl/reaction	125µl
KatG Primer & Probe Mix [KatG PP Mix] -5µl/reaction	125µl
inhA Primer & Probe Mix [inhA PP Mix] -5µl/reaction	125µl
PCR grade water	4ml
MTB Positive control [QS1] -10µl/reaction	125µl
Positive controls – rpoB, KatG & inhA - 10µl/reaction	125µl
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Storage

The content of the kit should be stored at –20°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours.

Intended Use

The HELINI MTB & MDR Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of MTB Complex and Multidrug resistance mycobacterium tuberculosis in Human biological samples.

The following MDR mutations will be detected (detects the presence of any mutations in the particular gene but does not distinguish between them)

MDR:

Isoniazid & Rifampicin resistance

rpoB (16 mutations)
katG [4 mutations]
inhA [3 mutations]

Product Use Limitations

- All reagents may exclusively be used in Molecular diagnosis.
- The product is to be used by personnel specially instructed and trained in Molecular diagnosis.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

Technical Assistance

For technical assistance and more information, please contact;

0091-9382810333
0091-44-24490433
helinibiomolecules@gmail.com

Dynamic range of test

Under optimal PCR conditions, kit has very high priming efficiencies of >95% and can detect less than 10 copies of target template.

Precautions

- Store positive material (Specimens, Standards or amplicons) separately from all other reagents and add it to the reaction mix in a separate facility.
- All the standards & specimens should be mixed & dispensed in extraction area.
- All the reagents including the NTC (except for standards & specimens) should be mixed & dispensed in pre-mix area.
- Use pipette tips with filters only.
- Always use disposable powder-free gloves

Introduction

HELINI MTB & MDR Real-time PCR Kit constitutes a ready-to-use system for the detection of MTB complex and Multidrug resistance mycobacterium tuberculosis using Real-time polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of mutant region from the MTB genome, and for the direct detection of the specific amplicon in fluorescence channel FAM. In addition, it contains an internal control amplification system as extraction control as well as to identify possible PCR inhibition. External mutant specific positive controls are supplied to validate.

Specificity

Primer and Probe have been designed for the specific and exclusive *in vitro* quantification of MTB Complex [*Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium canettii, Mycobacterium microti*.] The target sequence (MBP64) is highly conserved used for designing. The primers and probe sequences in this kit have 100% homology with a broad range of clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

Positive control
Multi drug and extended drug resistance mutant positive templates are provided with the kit. Include any one of them in every run.

Negative control
To confirm the absence of contamination, a negative control reaction should be included every time the kit is used. For this reaction, the RNAse/DNAse free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Internal Control template
When performing DNA extraction, it is often advantageous to have an exogenous source of nucleic acid template that is spiked into the lysis buffer. This internal control nucleic acid template is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and real-time PCR for the control template also indicates that PCR inhibitors are not present at a high concentration. The primer and probe present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the Internal control template does not interfere with detection of the pathogen target gene even when present at low copy number. The Internal control is detected through the HEX channel and gives a CT value of 21 +/-10.**Add 5µl of the internal control template to each sample. Do not add directly to the sample. Add to sample/lysis buffer complex**.

MTB detection Protocol

Things to do before starting
Before each use, all reagents need to be thawed completely, mixed by inverting several times and centrifuged briefly. Make sure that Positive and Negative control is included per qPCR run. Make sure that internal control template is added during DNA purification.

Detection Mix

Components	Volume
Probe PCR Master Mix	**10µl**
MTB PP Mix	**2.5µl**
IC PP Mix	**2.5µl**
Purified DNA sample	**10µl**
Total reaction volume	**25µl**

Centrifuge PCR vials briefly before placing into thermal cycler.
[Note: There should not be any bubbles in the reaction mix. Bubbles interfere with fluorescence detection.]

Negative Control setup
Include 10µl of nuclease free water

Qualitative Positive Control setup
Include 10µl of Positive control provided [QS1]

Amplification Protocol

	Step	Time	Temp
	Taq enzyme activation	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	56ºC
	Extension	20sec	72ºC

MTB = FAM channel

Internal Control = HEX Channel

Reading the graph:

Step-1
Select only test sample wells, select HEX/VIC/JOE channel and view the graph of internal control amplification. A successful amplification Ct value must be less than Ct 31. [Range 11 to 31].

This range indicates that test sample is purified well and there is NO PCR inhibition in the reaction. Any sample value goes beyond Ct value 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Only one exceptional case that Ct value beyond 31 can be accepted that the test sample is high positive for the MTB. Any test sample Ct value is beyond 31 must be re-purified and re-run the assay.

Step-2
Select the NTC and Positive control [Qualitative] or Standards wells [Quantitative], select FAM channel, view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC.

The PC or Standards must be amplified as per their copy numbers.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment.

PC justifies the reagents storage conditions and reaction parameters are as prescribed.

Step-3

In FAM channel, Select the NTC well and test sample well one by one, analyze the graph/amplification. Every time, select the NTC well and the test sample well, one by one analyze.

Test Sample	Negative control	Positive control	Internal Control	Interpretation
Positive	Negative	Positive	Positive	MTB DNA detected
Negative	Negative	Positive	Positive	MTB DNA Not detected. Sample does not contain detectable amount of MTB DNA.
Positive Or Negative	Negative	Negative	Negative	Experiment failed. PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.
Positive or Negative	Positive	Positive	Positive	Experiment failed. Reagent contamination. Repeat testing using fresh reagents.

Note:

Detection of the Internal Control in the JOE/HEX/VIC detection channel is not required for positive control and negative control.

MDR gene mutations:

Drug resistance – MTB-MDR			
RiF-R	rpoB	L511P CTG→CCG Q513K CAA→AAA Q513L CAA→CTA Q513P CAA→CCA D516V GAC→GTC	D516Y GAC→TAC S522L TCG→TTG S522Q TCG→CAG H526C CAC→TGC H526D CAC→GAC
		H526L CAC→CTC H526N CAC→AAC H526R CAC→CGC H526Y CAC→TAC S531L TCG→TTG	
INH-R	katG	S315I AGC-ATC S315N AGC-AAC	S315T AGC-ACC S315T AGC-ACA
	inhA	-15 C-T	-8 T-C -8 T-A

MDR Detection protocol:

Components	ropB	katG	inhA
Probe PCR Master Mix	10µl	10µl	10µl
ropB PP Mix	5µl	---	---
KatG PP Mix	---	5µl	---
inhA PP Mix	---	---	5µl
DNA purified	**10µl**	**10µl**	**10µl**
Total reaction volume	**25µl**	**25µl**	**25µl**

Include Positive and negative controls.

Amplification Protocol

	Step	Time	Temp
	Taq enzyme activation	15min	95ºC
40 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	60ºC
	Extension	20sec	72ºC

*** rpoB/katG/inhA = FAM channels**

Interpretation:

MDR:
Isoniazid & Rifampicin resistance
rpoB (16 mutations)
katG [4 mutations]
inhA [3 mutations]

Result interpretation:

ropB gene amplification indicates that test sample is Rifampicin [RMP] sensitive. [NOT resistance].

KatG and inhA gene amplification indicates that test sample is Isoniazid [INH] sensitive [NOT resistance].

No amplification of ropB/KatG/inhA indicates that test sample acquired MDR Resistance to particular gene.

Limitations

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

A false negative result may occur if inadequate numbers of organisms are present in the sample due to improper collection, transport or handling.

A false negative result may occur if an excess of DNA template is present in the reaction. If late CT or inhibition of the internal control is noted for a particular sample, purified DNA can be tested at 2 or more dilutions [e.g., 1:3 and 1:6) to verify the results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the pathogen's genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect the presence of the pathogen.

Manufactured and Marketed by

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