GeneFinder™
"SMART Solution for Better Life"

ISO 13485 Certified

TB & NTM Multiplex Real-time PCR Kit

REF **IFMR-07.02B100** Σ **100 tests/ kit**

Store at -20℃ or below.
Shelf life is 12 months after manufacturing.

CE-IVD

INTENDED USE

GeneFinder™ TB & NTM Multiplex Real-time PCR kit is designed for detection of DNA of *Mycobacterium tuberculosis* (TB) complex and NTM (nontuberculous *mycobacteria*). The product is intended for use in the diagnosis and monitoring of *M. tuberculosis* complex and NTM infections alongside clinical data of the patient and other laboratory tests.

KIT COMPONENT

TB & NTM Multiplex Real-time PCR kit	100 tests/kit
TB & NTM Reaction Mixture*	1,000 µL
TB & NTM Probe Mixture**	500 µL
TB & NTM Positive Control***	50 µL
TB & NTM Negative Control***	50 µL

*, TB & NTM Reaction Mixture; DNA polymerase, Uracil-N-glycosidase (UNG), Buffer containing dNTPs
**, TB & NTM Probe Mixture, Oligonucleotides for amplification and detection of target and plasmid for internal control
***, TB & NTM Positive Control; Clones for targets
****, TB & NTM Negative Control; Ultrapure quality water, PCR-grade

DESCRIPTION

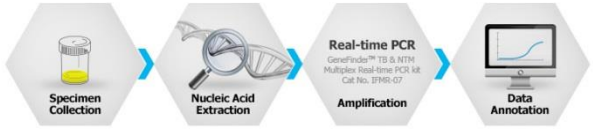
Tuberculosis(TB) is a disease caused by an infection with the bacteria *Mycobacterium tuberculosis* complex. It is highly contagious through the air, and can remain in an inactive state for years without causing symptoms or spreading to other people.

The non-tuberculous mycobacteria (NTM) refers to all the species in the family of mycobacteria that not cause tuberculosis but may cause human disease. Since Mycobacterium is difficult to culture and needs to be distinguished for diagnosis, it is highly important to be detected and differentiated based on PCR method.

WARNING AND PRECAUTION

- This product is designed for *in vitro diagnostic* use only.
- Do not use a kit after expiry date.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- Unnecessary repeated freezing and thawing will be occurred inaccurate results.
- Do not mix reagent from different batches of the kit.
- Do not modify the reagent/sample volume used in the test or use in a wrong way which is not recommended.

WORK FLOW



PROTOCOL

A. Specimen

This product is must be used with DNA extracted from human sputum or BAL.

B. DNA Extraction

It is recommended to use commercialized extraction kit such as QIAamp DNA blood mini kit (Cat. # 51304, Qiagen).

C. Reagent Preparation

Before setting up PCR, all components need to be thawed, gently mixed and centrifuged briefly to collect solution at the bottom.

1. Mix 10 µL of TB & NTM Reaction Mixture and 5 µL of TB & NTM Probe Mixture to prepare master mixture per each reaction (refer to the below). Prepare enough volume of master mixture for all the reactions plus extra to prevent pipetting error.
2. After mixing well, place 15 µL of master mixture into 96-well plate or PCR tube.
3. Add 5 µL of extracted DNA sample into 96-well plate or tube, then mix all components by pipetting. Proceed in the same way with other DNA samples, positive and negative control (Ultrapure quality water, PCR-grade).
4. Accurately close the tube with the cap or seal the 96-well plate.
5. Transfer the tubes or 96-well plate for test into the Real-time PCR and start for the amplification.

Component	**Per reaction (µL)**
TB & NTM Reaction Mixture	10
TB & NTM Probe Mixture	5
DNA sample or PC[1] or NC[2]	5
Total volume	20

[1], PC, positive control; [2], NC, negative control

(continued on reverse page)

D. Setting of Real-time PCR

1. Referring to the instrument manual, set on the dedicated software the parameters of thermal cycle.
2. Set up the PCR program and fluorescence as following, and then click the start “Run” button.

Temp.	Time	Ramping rate** (qTOWER^3)	Cycles
50℃	2 min	4 ℃/S	1
95℃	10 min	4 ℃/S	1
95℃	15 sec	4 ℃/S	40
60℃*	60 sec	2 ℃/S	

* Select “Collect Data” or “plate Read”
** For qTOWER3, set the Ramping rate as follows.

Target	Fluorescence				
	ABI	CFX96	Gentier 96E	qTOWER^3 Reporter dye	Gain
TB	FAM	FAM	FAM	FAM	3
NTM	JOE	HEX	JOE	HEX3	5
IC	Cy5	Cy5	Cy5	Quasar670	5
Reference dye ‘ROX’*					

Note:* *Applies to ‘ABI’ and ‘Gentier96E’ only.*

E. Analysis Setting

The values of fluorescence emitted by the specific probes and by the specific internal control probe in amplification reactions should be analyzed by the instrument software.
Click Analysis mode after completion and choose analysis setting from Amplification Plot.
Click “Edit Default Settings” to set threshold values as shown below.

Target	Threshold				Baseline	
	ABI	CFX96	Gentier 96E	qTOWER^3 *	Begin	End
TB	0.15	900	500	3	AUTO	
NTM	0.1	500	500	3		
IC	0.05	200	300	5		

Note : *Color compensation : Standard 1

F. Result Interpretation

Here are examples for result interpretation if the sample is positive /negative.

#	TB	NTM	IC	Assay Result
1	≤40	≤40	≤40	TB Positive or TB, NTM co-infection (only in case of NTM Ct < TB Ct)
2	≤40	≤40	U.D*	TB Positive
3	≤40	U.D	≤40	TB Positive
4	≤40	U.D	U.D	TB Positive
5	U.D	≤40	≤40	NTM Positive
6	U.D	≤40	U.D	NTM Positive
7	U.D	U.D	≤40	Negative (No Target)
8	U.D	U.D	U.D	Invalid (re-test)

* If the target DNA detected with high concentration in a sample amplification reaction, the internal control (IC) may results as Ct Undetermined (U.D). In case of CFX 96, U.D was displayed to N/A (Not applicable) and in case of qTower3 and Gentier 96E were displayed to No Ct and "-" each.

Quality control

#	TB	NTM	IC
PC	20.40±2	20.47±2	< 40
NC	U.D or N/A or ‘-’ or not Ct	U.D or N/A or ‘-’ or not Ct	< 40

TROUBLE SHOOTING

Problem	Possible Cause	Recommendation
No signal in all samples including positive control	Error in master mixture preparation	Check the dispensing volume during preparation of master mixture
	Inhibitors added	Repeat the extraction step with new sample
	Probe degradation	Use a new probe reagent
	Positive control degradation	Use a new positive control
	Omitted components	Verify each component, repeat the PCR mixture preparation
	Instrument setting error	Check the position setting for the positive control on the instruments. Check the thermal cycle settings on sample instrument
No signal in negative control	Carry-over contamination	Dispense carefully the sample, negative control and positive control
	96-well plate or tube error	Check the leaking of the plate or tube
	Tube cap/ sealing film error	Check the condition of closure for cap or sealing a film
	Reagent contamination	Repeat the test with new dispensing reagent
	Contamination of extraction or amplification area	Clean the instrument with disinfectant and replace with tubes and tips
Weak or no fluorescent signal in samples only	Poor DNA quality	Use recommended kit for DNA extraction and store extracted DNA at -20℃
	Insufficient volume of DNA	Repeat PCR reaction with correct volume of DNA
Weak or no fluorescent signal in positive control only	Probe degradation	Use a new probe and kit
Diverse intensity of fluorescent signals	Pipetting error	Make sure that the equal volume of reactants is added in each tube or plate
	Contamination in the outer surface of PCR tubes or plate	Wear gloves during the experiment

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