

**Viga MTB Molecular Diagnostic Kit****Store at -20 to -25°C****In darkness****100 rxn****Cat No: MD003054****25 rxn****Cat No: MD003057****By ROJE****Edition, 02/2022****2022 ROJETechnologies, all rights reserve****Kit Content**

Kit content	25 Preps	100 Preps
Pro MTB Mix	250µl	1000µl
Q-ROMAX, 4X	125µl	500µl
MTB Positive	40µl	150µl
Control		
RT-PCR Grade Water	40µl	150µl

Recommended Starting Material

Before starting the procedure, please study all safety guidelines in connection with the sample of MTB on this site <https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>.

Before starting any tests, each component must be melted, vortexed, and centrifuged briefly. Avoid repeated freeze-thaw cycles.

Before Start

Take out each component from the kit and place them on the bench top. Allow the

reagents to equilibrate to room temperature, then briefly vortex each tube for later use.

Buffer Preparation**Table 1:** preparation of components per single reaction

components	Volume
Q-ROMAX, 4X	5µl
Pro MTB Mix	10µl
Isolated DNA	5µl

Thermal Profile**Table 2:** Thermal profile for PCR reaction

Stage	Temperature	Incubation Time	Cycle Numbers
Pre-Denaturation	95 °C	3 min	1
Denaturation	95 °C	10 sec	45
Annealing and acquisition on channel	60°C	40 sec	

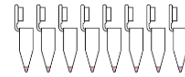
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Protocols

Thaw all reagents thoroughly at room temperature (15–25°C). When thawed, mix all reagents (by repeatedly pipetting up and down or by pulse vortexing) and centrifuge briefly. Work quickly and keep all reagents in the cooling block.

The volume of eluted sample DNA in this test should be 5µl. Prepare PCR reaction and then perform Real Time-PCR.

Step 1:
Equilibrate Q-Romax,4X and Pro MTB -Mix to room temperature



Step 2:
Add 5µl QD-Romax,4X in to clean microtube



Step 3:
Add 10µl Pro MTB Mix to the previous tube



Step 4:
Mix all reagents



Step 5:
Pulse Vortex the mixture



Step 6:
Centrifuge briefly



Step 7:
Add 5µl isolated DNA



Step 8:
Run the PCR program



Step 9:
Result interpretation

MTB (FAM)	IC (HEX)	Results
Ct ≤ 40 (+)	Not considered	Positive control*
-	Ct > 35 (+)	Negative control*
-	-	Invalid and not accepted

Figure 1: preparation of reagents, PCR run, and interpretation of results.