NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

SHENTEK Biofargo

www.biofargo.com
804-529-2296
contact@biofargo.com

SHENTEK

Mycobacteria DNA Detection Kit

User Guide

Version: A/1
For Research Use Only
Product No.:1503602
Reagents for 50 Reactions

Biofargo, Inc.

（IMPORTANT: Please read this document carefully before experiment.）

1. Product information

■ Product description

MycoSHENTEK® Mycobacteria DNA Detection Kit is used, together with MycoSHENTEK® Mycobacteria DNA Extraction Kit, to qualitatively determine whether there is Mycobacteria contamination in cells, cell culture-derived biologicals, etc., using real-time qPCR technology.

The integrated system has been fully validated at a detection limit of 10-100 CFU/mL to detect more than 100 different Mycobacteria species. The system contains dUTP, and if used with the UNG enzyme to prevent contamination and eliminate carry-over contamination.

Internal Control (IC) can be added to a PCR amplification reaction mixture to evaluate whether a test sample inhibits the amplification reaction and may result in false negative results. It can also be added to the sample before extraction to evaluate the extraction efficiency.

For extraction information, please refer to MycoSHENTEK® Mycobacteria DNA Extraction Kit User Guide (Product No.: 1503601).

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
MB qPCR Reaction Buffer	NNB011	400 µL × 1 tube	-20℃, protect from light
MB Primer & Probe MIX	NNC035	75 µL × 1 tube	
MB Internal Control (IC)	NNA037	600 µL × 1 tube	-20℃
MB Positive Control (PC)*	NNA041	500 µL × 1 tube	
DNA Dilution Buffer(DDB)	NND001	1.5 mL × 1 tube	

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Applied instruments, including but not limited to the following

- SHENTEK-96S Real-time PCR System
- CFX96 Real-Time PCR System
- 7500 Real-Time PCR System

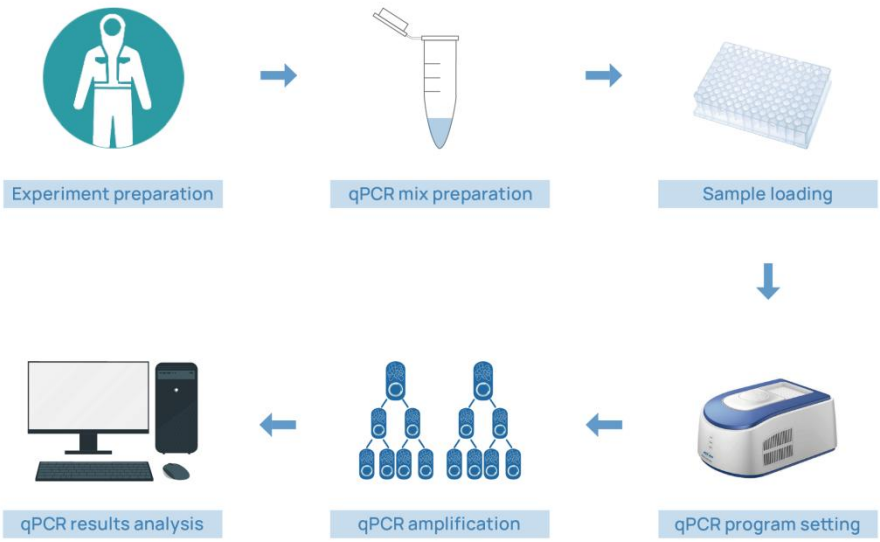
■ Required materials not included in the kit

- Nonstick, RNase-free microfuge tubes, 1.5mL, 2.0mL
- PCR 8-well strip tubes with caps or 96-well plates with seals
- Low retention filter tips 1000μL, 100μL, 10μL
- 75% Ethanol
- UNG enzyme (Please check the effective amount for best use.)

■ Related equipment

- Laminar flow cabinet or biosafety cabinet
- Benchtop microcentrifuge & PCR strip/plate centrifuge
- Microplate and micro test tube shaker
- Vortex mixer
- Real-time PCR System
- Pipettes, 1000μL, 100μL, 10μL

■ Workflow



2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, pipettes and tubes with UV for 30 minutes, and disinfect with 75% ethanol.
3. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.

■ qPCR MIX preparation

1. Determine the number of reaction wells according to the number of test samples and control samples with 2 replicates for each sample generally.

   Number of reaction wells = (1 Positive control (PC) + 1 No Template control (NTC) + 1 Negative control sample (NCS) + N Unknown samples)×2

2. Thaw the kit completely at 2-8°C or melt on ice, then prepare qPCR MIX according to the following table.

Table 2. qPCR MIX preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
MB qPCR Reaction Buffer	8 μL	264 μL
MB Primer&Probe MIX	1.5 μL	49.5 μL
IC*	0.5 μL	16.5 μL
Total volume	10 μL	330 μL
UNG enzyme (optional)	0.1 U	3.3 U

** If IC has been added during sample extraction, an equal volume of DNA dilution buffer should be added instead of IC to prepare qPCR MIX.*

■ qPCR Reaction MIX preparation

1. Vortex each solution and mix well. Then add the solution according to Table 3 to the plate layout of 96-well PCR reaction as shown in Table 4.

Table 3. qPCR Reaction MIX Preparation

PC (Positive control)	10μL qPCR MIX + 20μL PC
NTC (No Template Control)	10μL qPCR MIX + 20μL DNA Dilution Buffer
NCS (Negative control sample)	10μL qPCR MIX + 20μL of extracted NCS
Unknown sample	10μL qPCR MIX + 20μL of extracted unknown sample

Table 4.Example of 96-well plate layout

PC	PC				S1	S1						A
					S2	S2						B
					S3	S3						C
					S4	S4						D
					S5	S5						E
					S6	S6						F
					S7	S7						G
NTC	NTC				S8	S8				NCS	NCS	H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example shows one Positive control (PC), one No-template control (NTC),*

one Negative control sample (NCS), and eight unknown samples, with duplicate wells for each sample in qPCR analysis.

- *Adjust the layout according to the number of test samples to be run.*

2. Close PCR 8-well strip tubes with caps, or seal the 96-well plate with sealing film. Mix well in microplate or micro test tube shaker, then spin down the reagents for 10 seconds in centrifuge and place it in the qPCR instrument.

■ qPCR program setting

Please refer to the program setting as follows:

1. Run a new method program and select the quantitative PCR assay template.
2. Run a new Probe template, and type the name "Mycobacteria". Select FAM in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list. Select VIC in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK. Select the detection reference fluorescence as ROX (optional).
3. Set PCR cycling conditions:
   a. Set the cycling reaction volume to 30μL.
   b. Set the temperature and the time as following:

Table 5. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
UNG treatment	25℃	10:00	1
Activation	95℃	10:00	1
Denaturation	95℃	00:15	45
Annealing	60℃	00:30	
Extension	72℃*	01:30	

*Instrument will read the fluorescence signal during this step.

■ Result analysis

The following instructions apply only to the 7500 instrument with SDS v1.4.

1. Select the Results tab, then click Amplification Plot. In the Analysis Settings

window, enter the following settings:

a. Select **Manual Ct**.

b. In the **Threshold** field, enter 0.02.

c. Click **Analyze**, and check whether it is a normal amplification curve.

2. Select the **Results** tab, and click **Plate** tab, then set tasks for each sample type by clicking on the Task Column drop-down list and then click ▶:

a. NTC wells: target DNA detector task = NTC.

b. NCS, unknown samples wells: target DNA detector task = Unknown.

3. Select **File > > Export > > Results**. In the **Save as type** drop-down list, select **Results Export Files**, then click **Save**.

NOTE: If you use a different instrument or software, refer to the applicable instrument or software documentation. Usually real-time PCR instrument software automatically export the data report.

■ Acceptance Criteria

1. Guidance for the control samples as in the following table.

Table 6.Quality control samples results

Quality control samples	FAM	VIC
NTC	Ct≥40.00 or absence of specific amplification for duplicate runs	Ct<35.00 and normal amplification for duplicate runs
NCS	Ct≥40.00 or absence of specific amplification for duplicate runs	Ct<35.00 and normal amplification for duplicate runs
PC	Ct<35.00 and normal amplification for duplicate runs	Ct<35.00 and normal amplification for duplicate runs

The QC sample analysis shall be considered in relationship to LOD (Limit of Detection) based on method validation data.

2. Guidance for unknown samples as in the following table.

Table 7.Test sample results analysis

FAM	VIC	Conclusion
Ct<40.00 (at least one well) and normal amplification	Ct<40.00 and normal amplification for duplicate runs	Positive
	Ct≥40.00 or absence of specific amplification for duplicate runs	Presumptive Positive and presence of PCR inhibitors
Ct≥40.00 or non-specific amplification	Ct<40.00 and normal amplification for duplicate runs	Negative
	Ct≥40.00 or absence of specific amplification for duplicate runs	Not conclusive, and presence of PCR inhibitors

** If the VIC signal is inhibited, it is necessary to repeat the sample preparation or appropriately remove the inhibitors during the sample preparation and repeat the assay.*

Note: In an event that the sample is special, or some abnormalities occur, and results difficult to determine, please contact us for technical support.

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Support & Contact

SHENTEK

Biofargo, Inc.
www.biofargo.com
Address: 1716 E Parham Rd Richmond, Va, 23228, USA
E-mail: contact@biofargo.com
Phone: 804-529-2296