SHENTEK

Mycoplasma DNA Detection Kit (2G)

User Guide

Version: A/1
For Research Use Only
Product No.: 1509841
Reagents for 50 Reactions

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

1. Product information

■ Product description

MycoSHENTEK® Mycoplasma DNA Detection Kit (2G) is used, together with MycoSHENTEK® Mycoplasma DNA Extraction Kit (2G), to qualitatively determine whether there is mycoplasma, spiroplasma, or acholeplasma contamination in master cell bank, working cell bank, virus seed stock, or cell culture-derived products, etc. Validated according to USP 63, EP 2.6.7 and JP XVIII for mycoplasma detection with a detection limit of 10 CFU/mL.

The detection kit uses real-time PCR technology to detect more than 200 species of Mycoplasma, Spiroplasma and Acholeplasma with high specificity. It has been validated in a variety of matrices, as well as on non-mycoplasma species, production cells, and engineered bacteria and fungi.

Internal Control (IC) (2G) can be added to a PCR amplification reaction mixture to determine 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® Mycoplasma DNA Extraction Kit (2G) User Guide (Product No. 1509840).

■ Kit contents and storage

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

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
Internal Control (IC) (2G)*	NNA035	1 bottle	-20℃
Positive Control (PC) (2G)*	NNA039	1 bottle	
My qPCR Reaction Buffer (2G)	NNB004	400 μL × 1 tube	-20℃, protect from light
My Primer & Probe MIX (2G)	NNC065	75 μL × 1 tube	
DNA Dilution Buffer(DDB)	NND001	1.5 mL × 2 tubes	-20℃

** Reconstitute lyophilized Internal control (IC) (2G) in 600 μL of DNA dilution buffer, and lyophilized Positive control (PC) (2G) in 500 μL DNA dilution buffer, respectively.*

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

■ Applicable instruments, including but not limited to the following

- SHENTEK-96S Real-time PCR System
- CFX96 Real-Time PCR System
- 7500 Real-Time PCR System
- Lightcycler 480 Real-Time PCR System

■ Required materials not included in the kit

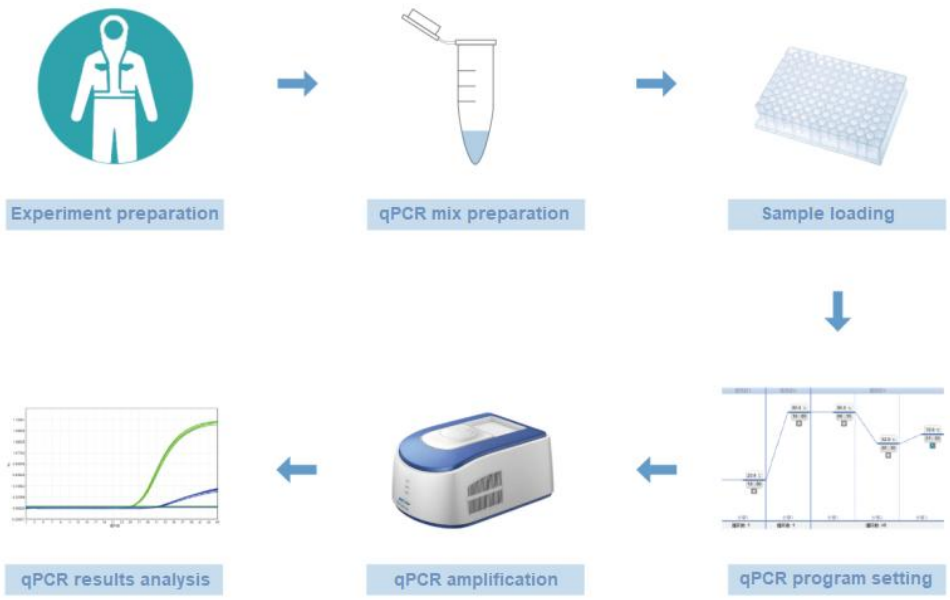
- Nonstick, RNase-free microfuge tubes, 1.5 mL, 2.0 mL
- PCR 8-well strip tubes with caps or 96-well plates with seals
- Low retention filter tips 1000 μL, 100 μL, 10 μL
- 75% Ethanol
- UNG enzyme (Please check the effective amount for best use.)

■ 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
- Microplats shaker
- Pipettes, 1000 μL, 100 μL, 10 μL

■ 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)**
My qPCR Reaction Buffer (2G)	8 μL	264 μL
My Primer & Probe MIX (2G)	1.5 μL	49.5 μL
Internal Control (IC) (2G)*	0.5 μL	16.5 μL
Total volume	10μL	330 μL
UNG enzyme	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 in each well

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

Table 4. Example of 96-well plate layout

NTC	NCS				S1	S1					PC	A
NTC	NCS				S2	S2					PC	B
					S3	S3						C
					S4	S4						D
					S5	S5						E
					S6	S6						F
					S7	S7						G
					S8	S8						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 “Mycoplasma-2G”. 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 30 μL.
   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	62℃	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. Control sample result analysis

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. Unknown sample result 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

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