Mycoplasma Rapid Detection Kit (qPCR)

Catalog Number: OPA-S101

Assay Tests: 25 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures.

IMPORTANT: Please carefully read this user guide before performing the experiment.

Product information

Mycoplasma Rapid Detection Kit (qPCR) is designed for the qualitative detection of mycoplasma DNA, it helps to detect mycoplasma contamination in cell banks, virus seed lots, cell and gene therapy products, raw materials, ancillary materials and other biopharmaceutical products. Validation was performed according to EP2.6.7 and JP XVIII Mycoplasma Nucleic Acid Amplification Test (NAT), (10 CFU/mL).

This kit only contains the mycoplasma DNA detection reagents, while the mycoplasma DNA extraction reagents are not included. Mycoplasma DNA Sample Preparation Kit (Magnetic Beads) is recommended for mycoplasma DNA extraction. For more extraction kit information, please refer to the Mycoplasma DNA Sample Preparation Kit (Magnetic Beads) User Guide (Cat. No. OPA-E101).

Mycoplasma DNA is detected by using TaqMan-qPCR assay. This kit can specifically qualitative detection for more than 250 mycoplasma species, *Acholeplasma laidlawii* and *Spiroplasma citri*. And it can avoid the disturbance of non-mycoplasma species, common engineered cells and cell culture medium. The UNG, which is contained in the master mix, can helps to avoid false positives in amplification. The internal control DNA in this kit can be used for monitoring both the extraction efficiency in the sample preparation stage and whether there is any inhibition for amplification in the PCR stage.

Contents and storage

The kit contains sufficient reagents to run 25 PCR reactions each with a final reaction volume of 30 μL.

Contents	Colors	Amount	Storage
2×qPCR Master Mix		400 μL×1	-30℃ to -15℃ Note: 2×qPCR Master Mix and Primer＆Probe Mix need protection from light.
Myco Primer＆Probe Mix		100 μL×1	
PC Powder		Lyophilized×1	
Purple-capped empty tube		Empty tube×1	
Internal Control DNA(IC)		300 μL×1	
DNA Dilution Buffer		1.5 mL×2	
DNase/RNase-Free Water		1.0 mL×1	

The unopened kit is stable for 18 months from the date of manufacture if stored at -30°C to -15°C.

Required materials not supplied

Instrument	Real-time PCR instrumentation
Consumables	96-Well Reaction Plate, Covers
	Nuclease-free, DNA-free aerosol-resistant pipet tips
	Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free) to prepare working solution.

Prepare the kit reagents

1. Thaw the reagents completely on ice, thoroughly mix reagents, and briefly centrifuge.
2. Preparation of Positive Control DNA(PC): Open the lid of PC Powder as the arrow shown on the lid, add 1.4 mL DNA Dilution Buffer and cover the lid, vortex it for 1 minute, transfer the solution to the Purple-capped empty tube, label the tube as Positive Control DNA.

   NOTE: The Positive Control DNA(PC) should be stored at -20℃, it can be divided into small portions to avoid freeze-thaw cycles.

Experiment control design recommendation

In order to make better judgments for unknown samples, sample controls are recommended as the following table. NEC and PEC should be prepared along with unknown samples using the OPA-E101 kit.

Controls	Sample Type	Function	qPCR Replicates
Positive Amplification Control (PAC)	Positive Control DNA	Monitor the amplification efficiency.	3
No Template Control (NTC)	No DNA	Monitor the contamination of qPCR reaction system preparation process and amplification.	3
Negative Extraction Control (NEC)	Extraction of Sample Matrix or Sample Dilution Buffer	Monitor the contamination of extraction process (extraction reagents, equipment, work areas and procedure).	3
Positive Extraction Control (PEC)	Extraction of Positive Control DNA	Monitor the efficiency of extraction and amplification.	3

Prepare the PCR reaction mix

1. Determine the number of controls and test samples whose DNA you will detect. Number of reaction wells is equal to three times the sum of **NTC, NEC, PAC, PEC, and test samples**.
2. Thaw reagents completely on ice, thoroughly mix reagents, and briefly centrifuge. Prepare a 2.0 mL tube for **Working Mix** (not add DNA template) using the reagents and volumes shown in the table below, thoroughly mix reagents, and briefly centrifuge. **IMPORTANT!** To compensate for pipetting losses, it is recommended that the **N** is equals to number of reaction wells plus 2 or 3.

Kit Reagents	Volume for 1 reaction	Volume for Working Mix
2×qPCR Master Mix	15 µL	15 µL×N
Myco Primer & Probe Mix	4 µL	4 µL×N
DNase/RNase-Free Water	1 µL	1 µL×N

3. Add **20 µL Working Mix** to each well separately.
4. Add template to the corresponding wells (reference to the table below). **Final volume** of PCR reaction is **30 µL**. It is recommended that the NTC, NEC, PEC, and PAC and test samples should be placed in different zones during the design and layout of the reaction wells to avoid cross contamination and inaccurate test results.

Reaction Well	Working Mix	Template	Total Volume
NTC (No Template Control)	20 µL	10 µL DNase/RNase-Free Water	30 µL
NEC (Negative Extraction Control)	20 µL	10 µL NEC purified DNA solution	30 µL
PEC (Positive Extraction Control)	20 µL	10 µL PEC purified DNA solution	30 µL
PAC (Positive Amplification Control)	20 µL	1 µL Internal Control DNA (IC) + 9 µL Positive Control DNA (PC)	30 µL
Unknown Sample	20 µL	10 µL Unknown sample purified DNA solution	30 µL

NOTE:

a. Template for Positive Amplification Control (PAC) should be prepared as shown in the table above, while all of other extracted samples should be added with 7 µL IC before extraction.

b. If the Internal Control DNA (IC) were not added into samples for extraction, you can replace 1 µL DNase/RNase-Free Water as 1 µL IC when preparing the working mix.

c. NTC don't need to add IC. If the working mix were added with IC, the reaction system for NTC should be prepared separately.

d. Set up a 96-well PCR plate using the example plate layout shown below.

5. Seal the plate with an optical film, then quick-spin with a centrifuge rotor that is compatible with 96-well plates.

Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC										PAC	
B	NTC										PAC	
C	NTC				S1	S1	S1				PAC	
D					S2	S2	S2					
E	NEC				S3	S3	S3				PEC	
F	NEC										PEC	
G	NEC										PEC	
H												

S=Sample; NTC=No Template Control; NEC=Negative Extraction Control; PEC=Positive Extraction Control; PAC=Positive Amplification Control.

NOTE: The plate layout is a suggested plate layout. Adjust the layout according to the number of test samples to be run.

Create the plate document and run the plate

The following instructions apply only to the ABI 7500 instrument. If you use a different instrument, refer to the applicable instrument guide for setup guidelines.

1. Create a new experiment, enter the experiment name in the Plate name field.
2. Select the **Quantitation Standard Curve** mode, **TaqMan** reagents, and **Standard** mode.
3. In the Plate Setup, create the targets as following steps:
   a. Enter the Target Name "**Myco**", select **FAM** in the Reporter Dye drop-down list. Select **(None)** in the Quencher Dye drop-down list.
   b. Enter the Target Name "**IC**", select **VIC** in the Reporter Dye drop-down list. Select **(None)** in the Quencher Dye drop-down list.
   c. Select **ROX** in the Passive Reference Dye drop-down list.
4. Set up the test samples and controls as shown in the Plate Layout.
5. Set up the qPCR reaction program according to following Table.
6. Select the reaction volume to 30 µL, click "Start Run" in the Run interface to start the qPCR run, and analyze the results after completion.

Step	Temperature	Cycles	Time	Signal Collection
1	37℃	1×	2 min	No
2	95℃	1×	30 sec	No
3	95℃	5×	10 sec	No
	65℃		30 sec	No
	72℃		30 sec	No
4	95℃	40×	10 sec	No
	64℃		30 sec	No
	72℃		30 sec	**Yes**

Analyze the results

After the qPCR run is finished, use the general procedure to analyze the results. For ABI 7500, set up the threshold value for FAM with 0.05 and auto baseline, while the threshold value for VIC is 0.06 and also select auto baseline. For other instruments, the setting of parameters should be adjusted according to the specific instrument user guide and software version.

The results judgement criteria for sample controls and unknown samples are shown in the following tables:

Sample Controls	FAM	VIC	Results
Positive Amplification Control (PAC)/ Positive Extraction Control (PEC)	Ct≤35	Ct≤35	Positive
Negative Extraction Control (NEC)	Undetermined/Ct > 35	Ct≤35	Negative
No Template Control (NTC)	Undetermined/Ct > 35	Undetermined/Ct > 35	Negative

NOTE: The judgement criteria for PEC (Positive Extraction Control) have the same as PAC (Positive Amplification Control).

Sample	FAM	VIC	Results
Unknown Samples	Ct≤35	Ct≤35	Positive
		Undetermined/Ct > 35	Extraction or Amplification inhibition, Retest
	Undetermined/Ct > 35	Ct≤35	Negative
		Undetermined/Ct > 35	Extraction or Amplification inhibition, Retest

NOTE: Replicates of qPCR are recommended for all the quality control samples and unknown samples. When qPCR replicates are 2 or 3, the result is trustable only if at least 2 replicates show the same results, otherwise it should be retested.