User Guide

Mycoplasma Detection Kit (qPCR)

Cat. No. D24011206 100 reactions of 20 μL final volume each

Store the kit at -25℃ to -15℃.

Version 01

Ducky Biotechnology Co., Ltd.

This product is for research purposes only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

Content

CHAPTER 1 Product description

1.1 Application

The Mycoplasma Detection Kit (qPCR) offers a rapid, reliable, and highly sensitive solution for qualifying biological samples against mycoplasma contamination. Utilizing real-time PCR (qPCR) technology, this kit amplifies a conserved genomic target specific to a broad range of Mycoplasma species, enabling precise qualitative detection.

1.2 Key Applications:

This kit is designed for the qualitative detection of mycoplasma, spiroplasma, and acholeplasma contamination in a wide variety of critical samples, including:

- Master and Working Cell Banks (MCB/WCB)
- Virus Seed Stocks
- Cell Culture-Derived Products
- Other Relevant Cell Culture Samples

For optimal performance, it is recommended for use in conjunction with the Sample Preparation Kit Ⅲ (Cat. No. S24020203).

1.3 Key Features & Benefits:

Broad Specificity: Detects over 90 species of Mycoplasma, Spiroplasma, and Acholeplasma with high specificity.
High Sensitivity: Achieves a sensitivity with a limit of detection (LOD) down to 10 CFU/mL.
Built-in Quality Control: An optional Positive Control (PC) is included.
Inhibition Monitoring: Add the PC to the PCR reaction to identify false-negative results caused by reaction inhibitors.
Extraction Efficiency Monitoring: Add the IPC prior to nucleic acid extraction to evaluate the efficiency of the extraction process.
Simple & Rapid: Streamlined workflow delivers reliable results in under 2 hours post-extraction.

CHAPTER 2 The Reagents and Samples

2.1 Required materials supplied

Table 1 Mycoplasma Detection Kit (qPCR) (Cat. No. D24011206)

Contents	Cap color	Amount	Storage
Mycoplasma qPCR Reagents			
Mycoplasma qPCR MIX		2×1.0 mL	−25°C to −15°C before first use, protect from light; 2°C−8°C for 1 week after first use.
PCR Negative Control		1×1.0 mL	−25°C to −15°C
Internal Positive Control （IPC）		1.0 mL	
Mycoplasma Positive Control (PC)		1.0 mL	
Dilution Buffer		7 mL	

Number of Tests: The kit contains sufficient reagents to run 100 PCR reactions each with a final reaction volume of 20 μL.

◆ This product is shipped on dry ice.
◆ This kit is stable until the expiration date printed on the label when stored at −25°C to −15°C.
◆ This kit is stable at +2°C to +8°C for 1 week.

Assay Time: Total time-to-result (without sample preparation): approximately **1.5 hours;**
Total time-to-result (with sample preparation): approximately **2 hours.**

2.2 Additional Equipment and Reagents Required

Application	Item
Miscellaneous	Standard laboratory equipment
	Disposable gloves
	Pipettes
	Nuclease-free, DNA-free aerosol-resistant, Low retention filter pipette tips
	Nonstick, Nuclease-free, Microfuge Tubes to prepare working solution, Dilutions.
	To minimize risk of nuclease contamination, autoclave all vessels and use alcohol wipes.
For nucleic acid isolation	Sample Preparation Kit Ⅲ (Cat. No. S24020203)
For the PCR	Laminar flow hood

workflow	Real-time PCR instrument for detection in FAM &ROX channel including accessories and consumables. We recommend the SLAN 96p.
	Vortex mixer
	PCR 8-well strip tubes with caps or 96-well plates with seals: plates are adapted to your PCR instrument.
	Centrifuge is adapted to your perforated plates or PCR 8-well strip tubes.

CHAPTER 3 Methods

3.1 Experiment Preparation

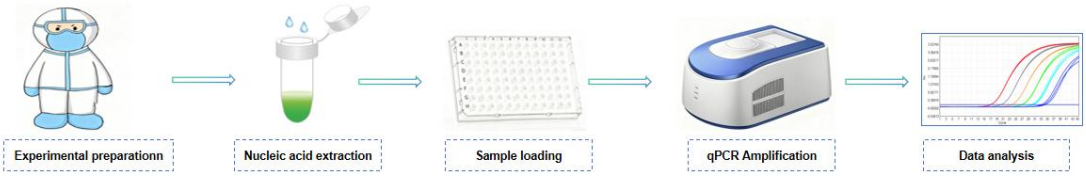
To avoid contamination, perform the workflow setup under DNA-free conditions. This includes:

- Wear appropriate protective eyewear, mask, clothing and gloves.
- Prepare and pipette all solutions with nuclease-free, DNA-free equipment and consumables.
- UV-treat or the laminar flow hood prior to pipetting.
- Use sterile single-use gloves and freshly laundered laboratory coats.
- Close tubes immediately after pipetting.
- Spatial segregation of the sequential workflow steps.

Rooms	Workflow Step
Sample preparation room	Extraction and purification of test samples, including preparation of recovery control sample.
Master mix preparation room	Master mix preparation and pipetting of PCR Negative Control to the NTC wells.
PCR room for setup and amplification run	Dilution and pipetting of samples and PCR Positive Control to the PCR plate. Running the PCR Instrument.

- In combination with this kit, a manual sample preparation with the Sample Preparation Kit Ⅲ (Cat. No. S24020203) is recommended. Note that due to different types of matrices (i.e. high protein amounts or very high DNA amount), the test samples should be appropriately diluted before running the sample preparation. For this purpose, use the Dilution Buffer provided in this kit (Nature Vial).

3.2 Workflow



3.3 Prepare qPCR Reactions

1. Thaw the kit at room temperature, mix thoroughly, and briefly centrifuge before use.
2. Then add 20μL qPCR MIX and 10μL solution (Negative control, Negative control Nucleic Acid Solution,Positive control Nucleic Acid Solution, Sample Nucleic Acid Solution) accordding to the table below to each tube or well to the plate of 96-well PCR reaction.

To prepare	In each tube or well
Negative control reaction	• Add 20 μL of Mycoplasma qPCR MIX • Add 10 μL of Negative Control
Unknown or sample reaction	• Add 20 μL of Mycoplasma qPCR MIX • Add 10 μL of Sample Nucleic Acid Solution
Negative control sample reaction	• Add 18 μL of Premix Solution • Add 10 μL of Negative Control (Nucleic Acid Solution)
Positive control reaction	• Add 20 μL of Mycoplasma qPCR MIX • Add 10 μL of Positive control (Nucleic Acid Solution)

3. 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. Be sure to remove any large air bubbles.

CHAPTER 4 Setup, run, and analyze samples with Software on the PCR Instrument

4.1 qPCR program setting

Mycoplasma detection assays are duplex assays, containing sample DNA and Internal Positive Control (IPC).

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-DuckyBio”. Select FAM in the Reporter Dye drop-down list and select (None) in the Quencher Dye

drop-down list. Select CY5 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:

qPCR running temperature and tim		
In this field…		Use these settings
Channel	Mycoplasma	FAM
	IPC	CY5
PCR	UDG[1]	Temp: 50 ℃ Time: 2:00
PCR	Hold	Temp: 95 ℃ Time: 5:00
PCR	Cycling (Standard Mode)	Cycles: 40 Temp: 95 ℃ Time: 0:15； Temp: 60 ℃ Time: 0:35, acquisition
Analysis	Mycoplasma	Automatic Baseline for Mycoplasmai and IPC.

[1]UDG enzyme is used in this kit to avoid the contamination of amplification products.

4.2 Analyze the results

1. Adjusting Analyze Parameters

- The instrument collects real-time fluorescence data during the experiment; when the experiment is over, the software automatically processes raw data in accordance with the analysis parameters of the project.
- Click Open Experiments button in the Experiment Home screen.
- The most frequently used analysis parameters of the current experiment is displayed in the analysis parameter table, which is below the Sample Information Table.
- Click the Analysis Parameters memu in the drop-down menu of Analysis to view all the analysis parameters. Choose Relative fluorescence method in the drop-down menu of Normolization algorithm. Click the Analysis menu to Analysis the result.
- Click Data in the menu bar to export data of this experiment. Data can be saved in formats of XLSX, CSV or TXT.

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.

CHAPTER 5 Result interpretation

5.1 Threshold line setting

The threshold line should be adjusted based on instrument noise to just exceed the vertex of the amplification curve of the Mycoplasma negative control sample. If the automatic threshold line of the instrument exceeds this point, it can still be used.

5.2 Internal Positive Control interpretation

For negative results, the Internal Positive Control Ct value should be ⩽ 35; for positive results, competition inhibition may cause no or poor values for internal controls.

For other qPCR instruments not mentioned above, the Ct value of IPC may be very different. For negative results, "Having an amplification curve of IPC" is generally regarded as a qualified standard; for positive results, competition inhibition may cause no or poor values for internal controls.

5.3 Experiment establishment conditions

For the qPCR instrument mentioned above, FAM channel shows No Ct value in NTC and NCS samples.

For other qPCR instruments not mentioned above, the Ct value range of each channel NTC is self-determined based on the results of multiple tests.

WARNING! GENERAL SAFETY.

Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

CHAPTER 6 Good laboratory practices

6.1 Work area setup and lab design

The sensitivity of this kit (and other PCR-based tests) enables amplification of minute quantities of DNA, necessitating precautions to avoid contamination of samples yet to be amplified .

Process samples carefully to prevent contamination by human DNA. Wear gloves at all times and change them frequently. Close sample tubes when not in use. Limit aerosol dispersal by handling sample tubes and reagents carefully.

6.2 Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
- Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
- Sample preparation and reaction setup.
- Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

6.3 Avoiding false positives due to cross-contamination

To avoid false positives due to cross-contamination:

- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative control, unknown, and positive control samples.

CHAPTER 7 Troubleshooting

Problem	Cause	Recommendation
Fluorescence intensity varies.	Some of the reagent is still in the upper part of the microwell or an air bubble is trapped in the microwell.	Repeat centrifugation, but allow sufficient centrifugation time so all reagent is at the bottom of the microwell and air bubbles are expelled.
	Skin oils or dirt on the surface of the microwell plate.	Always wear gloves when handling the multiwell plate.
Fluorescence intensity is very low.	Low concentration or deterioration of dyes in the reaction mixtures because dye was not stored properly.	1. Keep qPCR MIX reagents away from light. 2. Store the reagents at -25℃ to -15℃ and avoid repeated freezing and thawing.

	DNA is degraded during isolation or improper storage.	1. If possible, check DNA quality. 2. Store DNA samples at -25°C to -15°C.
	Pipetting errors and/or omitted reagents.	1. Check for missing reagents. 2. Check the pipetting procedure.
	Impure sample material inhibits reaction.	Dilute sample 1:10 to 1:1000and repeat the analysis.
Negative control sample gives a positive signal.	Contamination	Remake all critical reaction mixes. Be sure to use special pre-PCR setup working areas.
Δ Rn and Ct values are inconsistent with replicates	Evaporation of reaction mixture from some wells occurred because the optical adhesive cover was not correctly sealed to the reaction plate or due to over-drying the eluates in PrepSEQ™.	1. Select the Component tab. Confirm that affected wells generated significantly less fluorescence than unaffected replicates. 2. Check the amount of solution in each well of the reaction plate. Confirm that the wells affected by evaporation contained less solution than unaffected wells, and corresponded with the inconsistent results. 3. For subsequent runs, ensure that the optical adhesive cover is correctly sealed to the reaction plate.
	Incorrect volume of PCR reaction mix was added to some reactions.	1. Select the Component tab. Confirm that affected wells generated significantly less fluorescence than unaffected replicates. 2. Select the Spectra tab. Confirm that the wells with the incorrect volume of PCR reaction mix generated significantly different amounts of fluorescence than the unaffected wells. 3. For subsequent runs, ensure the correct volume of PCR reaction mix.

Jagged amplification plots	Weak lamp or incorrect replacement.	Replace the lamp or ensure that the existing replacement is correct.
No defined amplification plots	An incorrect detector was selected on the amplification plot. or An incorrect detector was applied to the reactions when setting up the plate document.	1. Ensure that the correct detector was selected on the amplification plot. 2. If the correct detector was not selected, then in the plate document, double-click a well to view the Well Inspector, verify that the detector settings are correct, and reanalyze.
There was a significant amplification curve but no Ct value could be obtained.	Incorrect passive reference was selected when setting up the plate document.	1. If you're using a qPCR instrument like the ABI 7500 that requires ROX background calibration, make sure to select "ROX" on the Reference Dye screen when performing qPCR amplification. 2.If you've already completed the experiment but selected "None" on the Reference Dye screen when performing qPCR amplification. then, in the Analysis Setting interface, uncheck "Use Default Settings" and "Automatic Threshold" respectively. Uncheck "Automatic Baseline." Adjust the "Automatic Threshold" value to 0.2. At the same time, the "Baseline Start Cycle" value should be "3" and "15." Then, after re-analysis and adjusting the appropriate threshold line, you should obtain a normal Ct value.
Wide variance of Ct values of replicate wells	Incomplete vortexing of samples.	Repeat reactions, ensuring that samples and standards are vortexed for 15-30 seconds.
The Ct Values of the start and curve is is abnormal	The disinfectant affected the PCR amplification	Do not use disinfectants during Sample preparation experiments so as not to affect the results.

CHAPTER 8 Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

·Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.

·Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).

·Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).

·Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.

·Handle chemical wastes in a fume hood.

·Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

·After emptying a waste container, seal it with the cap provided.

·Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

·Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

·IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! Reagent and Waste Bottle Safety. Reagent and waste bottles can crack and leak. Each bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.