Test for the detection of *Mycoplasma* by qPCR analysis

e-Myco™ VALiD-Q
Mycoplasma qPCR Detection Kit

RUO | REF 25245 | Σ 50 | -15°C ~ -25°C

BACKGROUND INFORMATION

Mycoplasmas are small, round or filamentous prokaryotic organisms which are a frequent contaminant of cell cultures. Mycoplasma depend on their hosts for many nutrients due to their limited biosynthetic capabilities. Up to 30~85% of cell cultures may be contaminated with mycoplasmas, the main contaminants being the species *M.orale, A.laidlawii, M.arginini* and *M.hyorhinis*. Although these mycoplasmas do not usually kill contaminated cells, they are difficult to detect and can cause a variety of effects on cultured cells, including changes in metabolism growth, viability and morphology, there by altering the phenotypic properties of the host cells. Many methods are available for detection of mycoplasma, including isolation in broth/agar culture, direct or indirect fluorescence staining, ELISA, immunostaining, direct or indirect PCR. Among those methods, direct PCR is the highly sensitive, specific and convenient method when the primer design is optimized.

The e-Myco™ VALiD-Q Mycoplasma qPCR Detection Kit is composed of a set of primers and probe that are specific for the highly conserved mycoplasma16S-rRNA coding region including *M.pneumoniae, M.argnini, M.hyorhinis, M.fermentans, M.orale* and *A.laidla*wii. The kit is designed to detect the presence of mycoplasma that might contaminate biological materials such as cultured cells. Also, the kit can detect mycoplasma within 90minutes sensitively up to 10 CFU/ml and includes internal control for verifying a qPCR run as well as positive control DNA.

PRINCIPLES

- The real-time qPCR(quantitative polymerase chain reaction) DNA amplification technology shows high sensitivity and specificity for direct detection of pathogen (antigen). iNtRON developed a novel platform technique about primer design called CLP™ (complementary locking primer) technology which provides flexibility in Tm (melting temperature) of primer design for optimization of reaction condition, and maximizes PCR specificity and sensitivity through the control of non-specific priming.
- The assay is a real-time PCR that discriminates mycoplasma in one reaction. The assay is composed of two principal steps: (1) extraction of DNA from specimens, and (2) amplification of the extracted DNA using 5′ nuclease fluorescent probe and specific primers pair. The assay amplifies two type specific regions: Mycoplasma(FAM) and IPC(HEX). An internal control is used to monitor the extraction process and to detect PCR inhibition.
- The internal positive control (IPC) has been introduced to the kit to verify the successful Real-time PCR reaction. The IPC is co-amplified with target band from test samples.

KIT CONTENTS

Contents	Composition
2X qPCR Master Mix Solution	• **Real-time PCR Reaction solution** < 0.01% dATP, dTTP, dGTP, dCTP < 0.01% Hot start PCR enzyme < 0.01% PCR additive materials
Detection Solution	• **Mycoplasma Detection solution** < 0.001% Primer/probe for Mycoplasma < 0.001% Internal control primer/probe set < 0.001% Internal control DNA
DNase/RNase Free Water	• **Ultrapure sterilized distilled water**
Positive Control (External PC)	• **Mycoplasma positive control** < 0.001 % Recombinant DNA contained 16S sequence of M. hyorhinis
Instruction Manual	

STORAGE AND STABILTY

- **Storage condition**: Store the product at -25~-15°C after receiving.
- **Expiration**: e-Myco™VALiD-Q Mycoplasma PCR Detection Kit can be stored for up to 12 months without showing any reduction in performance and quality under appropriates or age condition. The expiration date is labeled on the product box.

APPLICATION

The kit is used for the detection of mycoplasma species that are most commonly encountered in cell culture, including *M.peumoniae, M.arginini, M.fermentans, M.hyorhinis, M.orale,* and *A. laidlawii*. Furthermore, this kit can detect other various species of mycoplasma.

MATERIALS REQUIRED BUT NOT PROVIDED

- Realtime PCR Instrument
- Pipettes
- Centrifuge for micro-centrifuge tubes
- Disposable gloves
- G-spin Total Extraction Kit (Cat. 17045)
- Sterile pipette tip (with filter)
- Vortex mixer

PACKAGING INFORMATION AND STORAGE

Contents	Storage	Amount
2X qPCR Master Mix Solution	-25 ~ -15°C	520 µl x 1 vial (10 µl / test × 50 tests)
Mycoplasma Detection Solution	-25 ~ -15°C	260 µl x 1 vial
Positive Control (External PC)	-25 ~ -15°C	50 µl x 2 vials
DNase/RNase Free Water	-	1 ml x 1 vial
Manual	-	1 ea

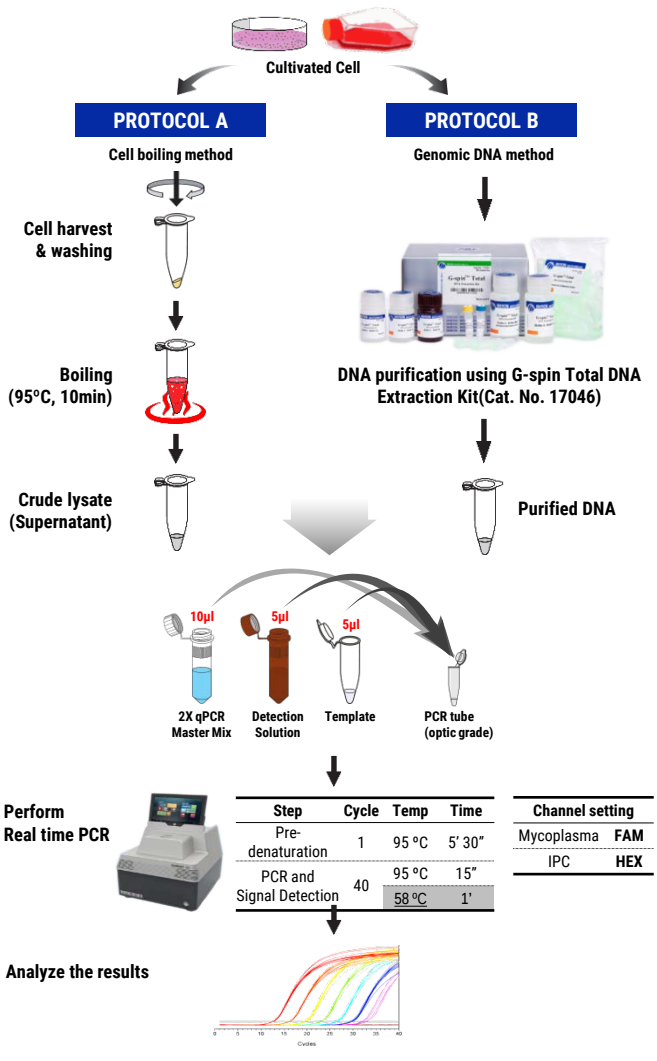
NOTICE

- To prevent contamination of mycoplasma DNA during experimental procedure, always wear gloves during sample preparation and PCR reaction setup.
- To avoid false positives, water used in PCR reactions can be UV-irradiated.
- If no internal positive control signal, it shows the problem during PCR process. Please re-test.
- If there is non-specific signal in negative control, it could be due to the contamination or over-used template. Please re-test with proper amount of template.

SHELF-LIFE

- 12 months from manufacturing date.
- Within 6 months after opening, within expiry date of the kit.

KIT WORKFLOW



PROTOCOL

You can use this protocol just for detecting the contamination of mycoplasma. However, if you want to perform genotyping for the detailed determination of species, please purify the genomic DNA of suspected Mycoplasma-infected cells using our G-spin Total DNA Extraction Kit (Cat.No.17046). You may use simply this protocol or your other general boiling methods.

[TECHNICAL TIP]

1. Use clean, disposable gloves when performing the assay and make sure that the work area is clean prior to starting the assay setup.
2. Keep your reagents and PCR mixture tubes on a cold block during reaction setup.
3. Use positive displacement pipettes.
4. The amplification and preparation areas should be physically separated.

PROTOCOL A : Using the Boiling Extract Method

1. **Prepare cell suspensions from the test cell culture in a 1.5 ml tube. Then count cell numbers by general counting methods. You need at least 5x10^4 cells per test.**
   Note 1: Harvest adherent cells with trypsin-EDTA solution using standard techniques. Pipette 1 ml of trypsin-EDTA treated adherent cells. Generally, with suspension cells you need not treat with trypsin-EDTA solution. We recommend that you count the cells. You should prepare at least 5x10^4 cells per test.
   Note 2: Strong mycoplasma infections are detected in as little as 10~100 cells, while weak infections require cells over 5,000~50,000 cells. You can dilute the template according to the infection rates you suspect
2. **Transfer the counted cells (over 5x10^4 cells) to a 1.5 ml tube. Spin the tube in a microcentrifuge for 10~15 seconds. Carefully decant the supernatant.**
3. **Resuspend the cells in 1 ml of sterile PBS or DPBS solution for washing.**
4. **Spin the tube in a microcentrifuge for 10~15 seconds. Carefully decant the supernatant.**
   Note : [Option] Repeat this wash step once more to reduce the unwanted PCR inhibition.
5. **Resuspend the cell pellets in 100 µl of sterile PBS or DPBS solution.**
   Note : If you want the best result, use of PBS solution is better than Tris (10 mM, pH 8.5), TE (10 mM Tris, 0.1 mM EDTA), or autoclaved DW.
6. **Heat the samples at 95 °C for 10 min, and vortex for 5-10 sec. Then, centrifuge for 2 min at 13,000 rpm with a tabletop centrifuge (at RT).**
7. **Transfer an aliquot of the boiled supernatant to a fresh tube. This supernatant will be used as the template in the PCR.**
8. **Prepare Detection Mix by dispensing components to each real-time PCR tube in the following manner**

Components	Master Solution (per test)
2X qPCR Master Mix Solution	10 µl
Mycoplasma Detection Solution	5 µl
Total volume	15 µl

9. **Fill up with the supernatant 5µl and Master Solution 15µl in the PCR tube.**
   Note 1 : For Negative Control : 5µl DNase / RNase Free Water
   Note 2 : For Positive Control : 5µl Positive Control
10. **After centrifugation, put them into a real-time PCR system and process reaction.**

Step	Cycle	Temp	Time
Pre-denaturation	1	95 °C	5′ 30″
PCR and Signal Detection	40	95 °C	15″
		58 °C	**1′**

Channel setting	
Mycoplasma	**FAM**
IPC	**HEX**

* Underline means signal detection step

PROTOCOL B : Using genomic DNA as a template

1. **Add 5µl of purified genomic DNA as a template using the G-spin Total DNA Extraction Kit (Cat. No. 17046), and then resuspend after adding Master Solution 15µl in the PCR tube.** **Note :** Appropriate amounts of DNA template sample: genomic DNA, 50 ng–100 ng
2. **Follow protocol A from step 10.**
   Note: Recommend to perform one negative control reaction by adding 5µl of sterile water. We recommend to add 5µl of control DNA for positive control reaction.

DATA VALIDATION

1. When the reaction is finished, put a cut-off value according to the below table.

Set Manual baseline	Threshold	Ct Cut-off Value
3 ~15	Auto	**Drop after 36 cycle**

❖ Manual setting of Threshold : Pull the threshold line into the graph. Adapt the threshold line to the 5~10% of saturation level of florescence signal of the positive control reaction.

2. Valid Results : Ct value of control should be as below table

Items	FAM	HEX	Items	FAM	HEX
Positive Control	18 ~ 22	22 ~ 25	Negative Control	<36	22 ~ 25

DATA INTERPRETATION

1. Expected Real-time PCR Data

No.	Samples	Mycoplasma (FAM)	IPC (HEX)	Interpretation
1	Positive control	+	+	**Valid**
2	Positive control	+	-	**Valid**
3	Positive Control	-	+	**Invalid (positive control degradation)**
4	Positive Control	-	-	**Retest (Reaction failure)**
5	Test 1	+	+	**Positive**
6	Test 2	+	-	**Positive (High conc. of Mycoplasma DNA)**
7	Test 3	-	+	**Negative (Mycoplasma Free)**
8	Negative control	-	+	**Valid**
9	Negative Control	+	+	**Contamination**
10	Negative Control\	-	-	**Retest (Reaction failure)**

❖ Ct value of IPC (HEX Channel) or clinical samples is usually between 15 and 35.
❖ Ct value of IPC over 25 may be resulted from competitive reaction with large amount of target DNA. That result is normal.

EXPLANATION OF SYMBOLS

LOT Batch number | RUO for research use only | REF Product number

Σ 50 Sufficient for 50 tests | Storage temperature limitation

Manufacturing date | Expire date

TROUBLESHOOTING GUIDE

Observation	Possible Cause	Recommendation
ΔRn ≤ No Template control ΔRn, and no amplification plot	Incorrect dye components chosen	Check dye component prior to data analysis
	Reaction component omitted	Check that all the correct reagents were added
	Degraded template or no template added	Repeat with fresh template
	Reaction inhibitor present	Repeat with purified template
ΔRn ≤ No Template control ΔRn, and both reaction show an amplification plot	Template contamination of reagents	Check technique and equipment to confine contamination. Use fresh reagents
		Repeat with aerosol barrier pipette tip after space cleaning
Amplification plot dips downwards	Ct Value less than 15, amplification signal detected too early	Reset the upper/lower value of baseline (two cycles lower than Ct Value). or repeat with diluted sample
Amplification plots is not within the log phase	PCR efficiency is poor	Re-optimization the reaction conditions
Ct value is higher than expected	Less template added than expected	Increase sample amount
	Sample is degraded	Evaluate sample integrity
Ct value is lower than expected	More template added than expected	Reduce sample amount
	Template contamination of reagents	Check technique and equipment to confine contamination. Use fresh reagents. Repeat with aerosol barrier pipette tip after space cleaning

Technical advice : +82-505-550-5600

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