

e-Myco™ VALiD-Q Real-time PCR Kit (ver.2.0)

Cat. 25246 50tests

DESCRIPTION

Mycoplasma is one of the smallest prokaryotic organisms and a common contaminant in cell cultures. Cells contaminated with mycoplasma experience inhibition of cell metabolism and growth, leading to changes in protein and gene expression. Generally, over 20 species of Mycoplasma and Acholeplasma are known to be major contaminants in cell cultures, with more than 5% to 35% of cell lines primarily contaminated by five species of Mycoplasma (*M. arginine*, *M. fermentans*, *M. hyorhinis*, *M. orale*, *A. laidlawii*). The contamination pathways for Mycoplasma can include culture additives used during the production process, the working environment, and the personnel involved, as well as cross-contamination from already contaminated cell lines.

Testing methods for detecting Mycoplasma are presented in domestic and international guidelines, with culture-based methods taking 28 days. These methods are not suitable for cell therapies produced in small quantities with a short shelf life, making the application of rapid testing methods (NAT) necessary. The e-Myco™ VALiD-Q Real-time PCR Kit (ver. 2.0) consists of a specific primer and probe set targeting the highly conserved Mycoplasma 16S rRNA coding region. This kit is designed to detect the presence of Mycoplasma that can contaminate biological materials such as cultured cells. Additionally, this kit can sensitively detect Mycoplasma down to 10 CFU/ml within 60 minutes and includes an internal control and positive control DNA for validating the real-time PCR execution.

PRINCIPLE

- **Amplification** : The extracted DNA is amplified using a 5' nuclease fluorescent probe and specific primer pairs.
- **Target Regions** : The assay targets two specific regions: Mycoplasma (FAM) and Internal Positive Control (IPC) (HEX).
- **Purpose of IPC** : The IPC is included in the kit to confirm the success of the Real-time PCR reaction and is co-amplified with the target band from test samples.

CONTENTS

- 2X qPCR Mix : 280 µl x 2 vials
- Detection solution : 140 µl x 2 vials
- Positive control (External PC) : 25 µl x 3 vials
- DNase/RNase free water (NC) : 1 ml x 1 vial

STORAGE CONDITION

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

SAMPLE PREPARATION

- Prepare cell suspensions from cell culture in a 1.5 ml clean tube. Then, count cell numbers by general counting methods. You need at least 5x10<sup>4</sup> cells per test.

**Note** : Harvest adherent cells with trypsin-EDTA buffer 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<sup>4</sup> cells per test

**Note** : 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.

- **Protocol A : Genomic DNA extraction method using a manual system**

We recommend using the G-spin™ Total DNA Extraction Kit (iNtRON, Cat No. 17045), which can extract genomic DNA from the pelleting cell.

- **Protocol B : Genomic DNA extraction method using a Automated system**

We recommend using the FaSTARprep96 Automated Nucleic Acid Extraction System (iNtRON, Cat No. IMC-FAST-P96D) and AutoXT PGS DNA/RNA Kit (iNtRON, Cat No. 17168), which can extract genomic DNA from the pelleting cell.

PROTOCOL

1. Prepare Detection Mix by dispensing components to each Real-time PCR tube in the following manner.

Contents	Sample	PC	NC
2X qPCR Mix	10 µl	10 µl	10 µl
Detection solution			
Sample	5 µl	-	-
Positive control	-	5 µl	-
DNase/RNase Free water	-	-	5 µl
Total volume	20µl	20µl	20µl

2. After centrifugation, put them into a Real-time PCR system and process reaction.

Step	Temp.	Time	Cycle	Channel	Target
PCR and signal detection	37 °C	2 min.	1	FAM	Mycoplasma
	94 °C	2 sec.	1	HEX	IPC
	94 °C	5 sec.	5		
	60 °C	10 sec.			
	94 °C	5 sec.			
	60 °C	10 sec.	45	← Signal detection step	

ANALYSIS

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

Target	Baseline	Threshold	Positive	Negative
Mycoplasma (FAM)	Auto	Auto	≤45	>45
IPC (HEX)			22 ~ 27	

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

**Note :** Ct value of IPC over 27 may be resulted from competitive reaction with large amount of target DNA. That result is normal.

TROUBLESHOOTING GUIDE

Observation	Possible Cause	Recommendation
$\Delta Rn \leq$ No Template control $\Delta Rn$ , 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
$\Delta Rn \leq$ No Template control $\Delta Rn$ , and both reaction show an amplification plot	Reaction inhibitor present	Repeat with purified template
	Template contamination of reagents	Check technique and equipment to confine contamination. Use fresh reagents
	Template contamination of 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
	More template added than expected	Reduce sample amount
Ct value is lower than expected	Template contamination of reagents	Check technique and equipment to confine contamination. Use fresh reagents.
	Template contamination of reagents	Repeat with aerosol barrier pipette tip after space cleaning

ORDERING INFORMATION

Product	Specification	Cat No.
G-spin™ Total DNA Extraction Mini Kit	50 col.	17045
	100 col.	17046
AutoXT PGS DNA/RNA Kit	48 tests	17168-48
	96 tests	17168-96
Miracle-AutoXT Automated Nucleic Acid Extraction System	-	IMC-NC15P LUS
FaSTARamp96 Real-time PCR System	-	IMC-FAST-A96

OVERVIEW OF MYCOPLASMA DETECTION

