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Myco-Qtech Kit

(Detection)

Quantity: 50 Reactions
Storage: -20 °C
Shipment: Dry ice
Cat. No.: LG9981

Myco-Qtech Kit (Detection)

Introduction

Mycoplasmas are one of the most common contaminants in cell cultures but their presence may go undetected for months. Mycoplasmas compete with the cells for the nutrients in culture media that affect virtually every aspect of the cell behavior and can cause significant errors in experimental and clinical settings particularly in biopharmaceutical production, cell therapy, and tissue engineering.

Furthermore, commonly used cell culture antibiotics including penicillin, streptomycin, and vancomycin are not effective against mycoplasma contamination; hence, frequent testing of mycoplasma is recommended in laboratories, especially when receiving new cell or virus cultures, as well as before each cryopreservation event. For continuous cell lines (Hela, HEK293, CHO, etc.), monthly testing is the best method to control potential spreads of contamination.

Product Description

Livogen Myco-Qtech Kit (Detection) offers a quick and sensitive strategy based on Real-Time PCR (commonly known as quantitative PCR or qPCR) for detecting Mycoplasma contamination in cell cultures and other cell culture derived biologicals. This kit detects over 60 species/strains of Mycoplasma, Acholeplasma, Spiroplasma and Ureaplasma including the eight species most likely to afflict cell cultures: *M. arginini, M. fermentans, M. hominis, M.hyorhinis, M. orale, M. pirum, M. salivarium, and A. laidlawii.*

It worth noting that the Myco-Qtech Kit (Detection) has been tested and optimized for R&D use and is not recommended for clinical application.

Kit Component

Component	Volume	Storage
Myco-Q Master Mix (2X)	500 µl	-20°C
Myco-Q Primer Mix	150 µl	-20°C
Myco-Q Positive Control	300 µl	-20°C
Myco-Q Negative Control	150 µl	-20°C

Shipping and Storage Condition

The Myco-Qtech Kit (Detection) is shipped on dry ice and should be stored at -20°C in a constant-temperature freezer.

Equipment Required (not included)

1.qPCR device with filters for the detection of the fluorescence dyes

2. PCR reaction tubes for the specific qPCR device

3. 0.5 ml and 0.2 ml sterile microcentrifuge tubes, DNA and RNA free

4. Pipettes with corresponding filter tips (10, 100, 1000 µl)

Sample Preparation

1. The cells must have been grown without antibiotics for at least 3 subcultures.

2. The cells should remain in culture for at least 48-72 hours prior to Mycoplasma detection.

3. Collect the sample once the cells have reached at least 80% confluence.

4. DNA can be isolated using Myco-Qtech Kit (Extraction) or any DNA extraction method/kit that may not interrupt the Real-Time PCR procedure.

Precaution for qPCR

1. It is highly recommended to prepare the qPCR reactions in a laminar flow hood.
2. Use disposable powder-free gloves.
3. Work quickly on ice or in the cooling block.
4. Thaw all components thoroughly at room temperature.
5. Keep reactions and components capped and protected from light.
6. The positive controls should be prepared carefully for avoiding contamination of negative control and test samples.
7. In order to increase the accuracy of results, it is recommended to perform the tests in duplicate or triplicate.

qPCR Procedure

1. Prepare the reaction mixture in qPCR as follows:

Component	Positive Control	Negative Control	Spiked Sample	Test Sample
Myco-Q Master Mix (2X)	10 µl	10 µl	10 µl	10 µl
Myco-Q Primer Mix	3 µl	3 µl	3 µl	3 µl
Myco-Q Negative Control	—	7 µl	—	—
Myco-Q Positive Control	7 µl	—	—	—
Test Sample (DNA)	—	—	—	7 µl
Spiked Sample (DNA)	—	—	7 µl	—
Total Volume	**20 µl**	**20 µl**	**20 µl**	**20 µl**

2. Place the tubes in qPCR machine.

3. Use the following protocol for qPCR:

Step	Process	Temperature	Time	Cycle
1	Initial Denaturation	95 °C	10 min	1
2	Denaturation	95 °C	20 sec	40
	Annealing	63 °C	15 sec	
	Extension	72 °C	15 sec	
3	Melting Curve Analysis	67-95 °C	—	—

Specificity

The Myco-Qtech Kit (Detection) can detect more than 60 different Mycoplasma species and does not detect other genera or cell-line DNA.

Acceptance Criteria

Ct	Tm (°C)	Result
≤ 35	80-85*	Myco +
≥ 35	ND** or < 80 or > 85	Myco -

* The values are specific for the mycoplasma strain commonly used. Based on the detected mycoplasma strain, it may differ slightly.

**Not Detected.

Interpretation of Results

The acceptance criteria are based on our current knowledge of assay performance in detection of Mycoplasma recovered from a wide variety of test sample matrices. We recommend that you qualify and validate the assay internally using samples that are specific to your process and manufacturing environment (raw materials, bioreactor or cell line samples) in order to verify that these criteria are appropriate. For specific sample types, it may be necessary to make slight adjustments to the acceptance criteria based on specific results. Our research team at Livogen Pharmed Co. can provide you with one-on-one support during this process.

Results Analysis

Positive Control

The acceptance criteria should be met for positive control amplification curve and melting temperature. Amplification out of this range suggests a failure in Real-time PCR procedure.

Negative Control

In ideal circumstances, the negative control should show a flat line (negative result).

- Amplification curve observation at Ct > 35 is not valid.
- Non-specific melting temperature (< 80 °C or > 85 °C) and low peak of melting curve is not valid.

Spiked Sample

The acceptance criteria should be met for spiked sample amplification curve and melting temperature. Amplification out of this range suggests a failure in DNA extraction procedure.

Test Sample Evaluation

The test sample is positive for Mycoplasma, if the corresponding fluorescence accumulation curve cross threshold at Ct value ≤35 with specific melting temperature (80-85 °C).

- Amplification curve observation at Ct > 35 is not valid.
- Non-specific melting temperature (< 80 °C or > 85 °C) and low peak of melting curve is not valid.
- Amplification curve observation at Ct ≤ 35 with non-specific melting temperature and low peak of melting curve is not valid.

Troubleshooting

Observation	Possible Cause	Action
No positive-control or target specific SYBR® Green dye signal is detected in inhibition control and/or positive-control wells	Improper storage of Myco-Q Master Mix	Repeat the assay using properly stored assay components.
	Improper storage of target-specific Myco Primer Mix	Avoid freezing and thawing assay components. Protect Myco-Q Master Mix from light.
	Pipetting error (no premix solution added)	Repeat the assay. Make sure to pipet premix solution into all wells.
	Pipetting error (no positive control added)	Repeat the assay. Make sure to pipet positive control into all positive-control wells.
Target-specific signal is detected in negative-control wells	Carryover contamination	Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. If the negative control continues to show contamination, repeat the assay using a new kit. If the negative control continues to show contamination, contact your Application Specialist.
	High level of nonspecific product formation	Check the dissociation curve to confirm. Repeat the assay using properly stored assay components. Avoid freezing and thawing assay components. Protect Myco-Q Master Mix from light.

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