

Product Name

Name: MycoEZ, Mycoplasma qPCR Kit

Cat. No.: AC5010-0025

Size: 1 kit, 25 reactions

Package Specification

Component	Cap color	Included volume
Lysis Buffer	Blue	200 µl
20X TaqMan probe-FAM	Green	12.5 µl
2X Probe qPCR Master Mix	Brown	125 µl
Positive Control, 1000 copies/µl	Orange	18 µl
Negative Control	Yellow	18 µl

Introduction

The MycoEZ, Mycoplasma qPCR Kit enables rapid and highly sensitive screening and detection of mycoplasma - a common and dangerous contaminant of cell cultures and cell culture-derived biologics. This kit is designed to identify 14 mycoplasma genus species (table 1) in a single PCR reaction, utilizing real-time quantitative polymerase chain reaction (qPCR) and probe fluorescence labels. This creates a qPCR assay with enhanced sensitivity and specificity designed with a detection limit of 45 copies.

The MycoEZ, Mycoplasma qPCR Kit includes sample lysis buffer, a TaqMan probe labeled with FAM (Excitation 490 nm/Emission 520 nm), a probe qPCR master mix, as well as positive and negative controls.

Storage and Stability

Store all components at -20°C. The product is **light-sensitive** and therefore should not be left in the light. Avoid repeated freeze/thaw cycles. When in use, always keep kit vials on ice.

Procedure**Test sample collection and preparation:**

1. Aspirate 50 µl cell culture medium to a 1.5 ml eppendorf tube and centrifuge at 4,800 rpm or 2,000 xg for 3-5 minutes.
2. Carefully remove the supernatant (~50 µl) while preserving the pellet integrity.
Note: The pellet may not be easily visible at the bottom of the tube.
3. Resuspend the pellet with 8 µl of Lysis Buffer (Blue).

4. Pipette up and down five times to completely lyse the pellet.
5. The lysate can be subjected to qPCR for amplification.

Prepare the qPCR reaction:

Component	Test sample	Positive control	Negative control
2X Probe qPCR Master Mix (Brown cap)	5 µl	5 µl	5 µl
Nuclease-Free water	2.5 µl	2.5 µl	2.5 µl
20X TaqMan probe-FAM (Green cap)	0.5 µl	0.5 µl	0.5 µl
Digested lysate	2 µl	--	--
Positive or Negative control	--	2 µl	2 µl
Total	10 µl	10 µl	10 µl

qPCR Program:

Step	Temperature	Time	
Hold	95°C	1~2 min	polymerase activation
Cycle (35x cycles)	95°C	3 sec	denature
	60°C	30 sec	anneal/ extend

data collection: FAM labeled (Excitation 490 nm/Emission 520 nm)

Data analysis:

1. Manually set the threshold to 0.2.
2. Negative Control: Non detectable; Positive Control: Ct 28 ± 1.
3. Determine the Ct value for each sample.

Precaution and Disclaimer

For research use only, not for clinical diagnosis, and treatment.

Table 1: Mycoplasma species can be detected by MycoEZ, Mycoplasma qPCR kit:

<i>Mycoplasma arginini</i>	<i>Mycoplasma hyorhinis</i>
<i>Mycoplasma arthritis</i>	<i>Mycoplasma orale</i>
<i>Mycoplasma bovis</i>	<i>Mycoplasma pirum</i>
<i>Mycoplasma capricolum</i>	<i>Mycoplasma pneumoniae</i>
<i>Mycoplasma fermentans</i>	<i>Mycoplasma pulmonis</i>
<i>Mycoplasma hominis</i>	<i>Mycoplasma salivarium</i>
<i>Mycoplasma hyopneumoniae</i>	<i>Mycoplasma yeatsii</i>