

Product Name

Name: MycoEZ™ Mycoplasma qPCR Kit

Cat. No.: M5010-0025

Size: 25 preps

Product Description

The MycoEZ™ Mycoplasma qPCR Kit allows for quick and sensitivity detection of mycoplasma contamination in cell cultures.

The Mycoplasma species that can be detected by MycoEZ™ Mycoplasma qPCR Kit are:

Mycoplasma Group	Strains	
<i>Mycoplasma spp.</i>	<i>M. arginini</i> <i>M. arthritidis</i> <i>M. bovis</i> <i>M. capricolum</i> <i>M. fermentas</i> <i>M. hominis</i> <i>M. hyopneumoniae</i>	<i>M. hyorhinis</i> <i>M. orale</i> <i>M. pirum</i> <i>M. pneumonia</i> <i>M. pulmonis</i> <i>M. salivarium</i> <i>M. yeatsii</i>

The components included in the kit are listed in the following table.

Component	M5010-0025 (25 preps)
MycoEZ™ Lysis Buffer A (Blue)	200 µL
MycoEZ™ 2X Probe Fast qPCR Master Mix (Brown)	125 µL
MycoEZ™ Positive Control 1000 copies/µL (Orange)	18 µL
MycoEZ™ Negative Control (Yellow)	18 µL
MycoEZ™ 20X TaqMan Assay Dye (Green) FAM labeled (Ext.~490 nm/ Emi.~520 nm)	12.5 µL
Nuclease-Free Water (Translucent)	1 mL

Procedure

(A). Digest Cell Culture Medium

- (1) Aspirate 50 µL cell culture medium to a 0.5 mL Eppendorf tube and centrifuge at 2,000 xg for 3 minutes (mini centrifuge).
- (2) Discard the supernatant (~50 µL) with a pipette tip carefully, and the pellet might be "INVISIBLE" at the bottom of the tube.
- (3) Add 8 µL MycoEZ™ Lysis Buffer A to the bottom of the tube.
- (4) Pipette up and down 5 times to lyse the pellet completely and then prepare the qPCR reactions.



(B). Prepare the qPCR reactions:

10 μ L per qPCR reaction

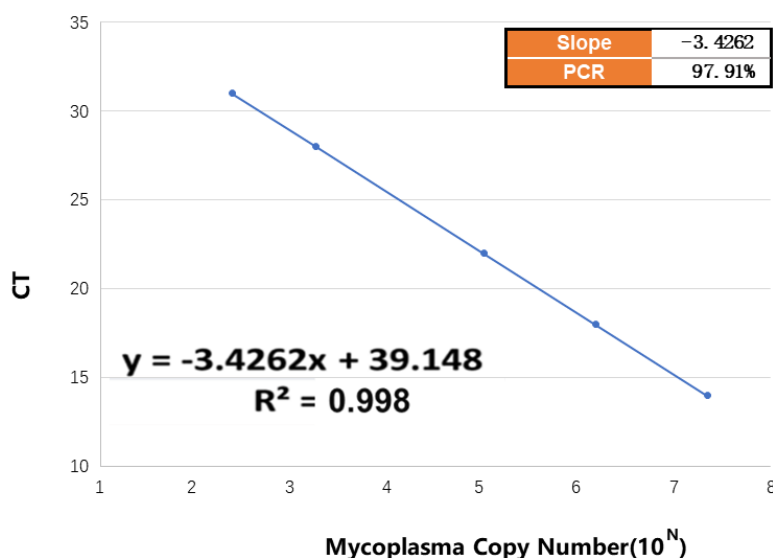
Component	Volume for one 10 μ L reaction		
	Test (μ L)	Positive Control (μ L)	Negative Control (μ L)
Nuclease-Free Water	2.5	2.5	2.5
MycoEZ™ 20X TaqMan Assay Dye	0.5	0.5	0.5
MycoEZ™ 2X Probe Fast qPCR Master Mix	5	5	5
Digested lysate	2	0	0
MycoEZ™ Positive Control	0	2	0
MycoEZ™ Negative Control	0	0	2
Total volume (μ L)	10	10	10

(C). Perform the qPCR Program:

Step	Time	Temp.	Data Collection	Note
Hold	1 min	95°C		★ Polymerase Activation
Cycle (35)	3 sec	95°C	■	Denature / Anneal / Extend
	30 sec	60°C		

★ : Polymerase Activation must be more than 1 min.

■ : FAM labeled (Ext.~490nm / Emi.~520nm)



Limit of Detection(LOD) : 45 copies

Limit of Quantification(LOQ) : 200 copies

Less than 45 copies is not suitable for this qPCR assay



(D). Analyze the data using the *Mycoplasma Copy number Calculator* (name of the web site):

- (1) Set the Threshold manually to 0.2
- (2) Negative Control = Non detectable; Positive Control (2000 copies) = Ct 28 ± 1
- (3) Determine the Ct value of each sample (Calculate the copy number)
- (4) Mycoplasma Copy number Calculator website link:

<http://www.xpbiomed.com/newsinfo/7178381.html>

Fill in the sample Ct, and the mycoplasma copy number (copies/uL) will be calculated automatically.

支原体拷贝数计算器/Mycoplasma Copy number Calculator	
Fill in the sample Ct (Y)	请输入
Mycoplasma Copy number (X)	请输入
自动计算 不用点	

Storage and shelf life

All components of the kit are stored at -20°C.

Shelf life: 24 months from date of manufacture.

Manufacturer

Shanghai Dr. Cell Co., Ltd.

Issue Date

April 2024

Precaution and Disclaimer

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

