

# WizDx™ CrystalMix Mycoplasma

## INTENDED USE

WizDx™ CrystalMix Mycoplasma is an in vitro diagnostic real-time PCR test for the qualitative detection of *Mycoplasma spp.* in extracted DNA of cell cultures and other cell culture-derived biologicals. WizDx™ CrystalMix Mycoplasma is for research use only.



## PRINCIPLES OF THE TEST

WizDx™ CrystalMix Mycoplasma combines all reagents necessary for successful Real-time PCR in a convenient individual aliquot and lyophilized in an 8-strip qPCR tube. Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target-specific probes for the detection of 16s rRNA gene in extracted DNA of cell cultures and other cell culture-derived biologicals. The probes are labeled with fluorescent reporter and quencher dyes. Probes specific for *Mycoplasma spp.* 16s rRNA are labeled with the fluorophore FAM. The probe is specific for the Internal Positive Control (IPC) and is labeled with the fluorophore Cy5 to monitor for PCR inhibition and to validate the quality of the sample and detection result.

## KIT STORAGE AND STABILITY

- Store at 4 - 25°C, **Do not freeze CrystalMix.**
- **Do not use it once the cone-shape mix shrinks as a dot form. It was damaged by rehydration.**
- Expires 12 months from the date of manufacture.

## KIT CONTENTS

Component	Amount	Cap Color
Mycoplasma CrystalMix	96 tubes	
Mycoplasma Positive control *	1 vial	
Deionized Sterile Water *	1 vial	

\* Before using the positive control, add 200µl of Deionized Sterile Water and dissolve sufficiently.

\* Positive Control and Deionized Sterile Water should be stored and shipped at room temperature. Once opened, please store them at -20°C.

## REAGENT AND EQUIPMENT TO BE SUPPLIED BY THE USER

- Real-Time PCR System
- Micropipette & sterile pipette tips
- Vortex mixer & microcentrifuge
- Protective ware & disposable gloves

## TEST SAMPLE

- Cell cultures and other cell culture-derived biologicals

## QUALITY CONTROL

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizDx™ CrystalMix Mycoplasma kit is tested against predetermined specifications to ensure consistent product quality.

## WARNINGS AND PRECAUTION

- For Research use only.
- For single use only. Do not reuse.
- Do not drink Deionized Sterile Water.
- Carefully read this instruction before use.
- Do not use any reagents after the expiration date.
- Always wear personal protective equipment (gloves, mask, etc.) when handling biohazardous agents in compliance with relevant regulations.
- Always use sterile, filtered pipette tips.
- Take care of the handling of samples to minimize the risk of infection.
- Dispose of waste in compliance with relevant regulations after the test.

## PROTOCOL

### [Step 1] DNA Preparation

#### 1) Boiling extraction method (enrichment culture sample)

1. Transfer 1mL of the enrichment sample to a microtube.
2. Remove the supernatant by centrifugation at 13,000 rpm for 10 minutes.
3. Add 500µL of distilled water to loosen the pellet.
4. Remove the supernatant by centrifugation at 13,000 rpm for 10 minutes.
5. Add 100µL of distilled water to dissolve the pellet.
6. Heat in a heating block or water bath at 100°C for 10 minutes.
7. Centrifuge at 13,000 rpm for 3 minutes.
8. Transfer the supernatant to a new microtube and use it as Sample DNA.

#### 2) Boiling extraction method (separate culture sample)

1. Take cultured colonies and suspend in 100 µL of distilled water.
2. Heat in a heating block or water bath at 100°C for 10 minutes.
3. Centrifuge at 13,000 rpm for 3 minutes.
4. Transfer the supernatant to a new microtube and use it as Sample DNA.

#### 3) Use of DNA extraction kit

For best results, we recommend using the following extraction kits.


- WizMag™ BMC DNA Kit (REF. W7030, Wizbiosolutions Inc.)
- WizPrep™ gDNA Mini Kit (Cell/Tissue) (REF. W71060, Wizbiosolutions Inc.)

### [Step 2] Prepare PCR Reaction

1) Prepare the PCR reaction as the following table.

Component	Sample	PC *	NTC *
Mycoplasma CrystalMix	1 tube	1 tube	1 tube
Sample (DNA)	20 µL	-	-
Mycoplasma Positive Control	-	20 µL	-
Deionized Sterile Water	-	-	20 µL
Total	20 µL	20 µL	20 µL

\* PC: Positive control, NTC: Non-template control

 **To avoid contamination, close the cap immediately after placing the sample in the tube.**

2) Vortex for 3 - 5 sec. and briefly spin down.

3) Insert the CrystalMix tube into the Real-Time PCR System.

### [Step 3] Run Real-time PCR

1) Prepare the Real-time PCR program set-up as the following table.

Step	Temp.	Time	Cycle
UDG Treatment (Carryover prevention)	50 °C	180 sec.	1
Pre-Denaturation	95 °C	180 sec.	1
Denaturation	95 °C	10 sec.	40
Annealing (Probe detection)	64 °C	30 sec.	

2) Set up the threshold and baseline of the fluorescence probes as follows:

PCR System	Fluorescence	Threshold	Baseline	
			Begin	End
CFX96™	FAM	100	Auto	
	Cy5	50		
QuantStudio5	FAM	Auto		
	Cy5			
CLEO™ Q16	FAM	Auto		
	Cy5			

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## [Step 4] Analysis of Results

For interpretation, please refer to the Interpretation table.

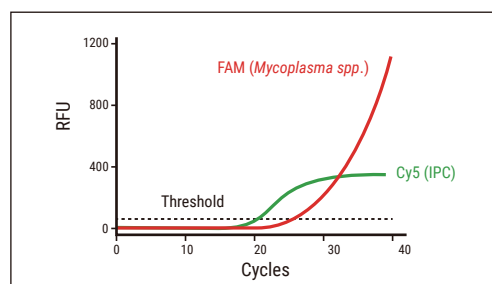
- Cut-off value of sample: **Ct <40**

Test	FAM	Cy5	Results
#1	+	+/-	<i>Mycoplasma spp.</i> detected
#2	-	+	No detected
#3	-	-	Invalid (Retest)

\* Cy5 (Internal Control; IC) shall always be amplified. If the concentration of nucleic acids in the sample is high, the IC signal may be inhibited or offset. In case the target gene signal is strong, Cy5 (Internal Control; IC) could be negative.

\* If you want to check the IC, it is recommended to dilute the sample and retest.

## EXAMPLE OF THE RESULTS



## PERFORMANCE EVALUATION

### 1. Limit of Detection (LoD)

Results were statistically analyzed by probit analysis. LoD is determined as the lowest DNA concentration that produced at least 95% positive results. It has been concluded that the limit of detection (LoD) is valued at 5 copies/uL (24 repeats).

### 2. Specificity

No.	Species	Detection
1	<i>Acholeplasma laidlawii</i>	0
2	<i>Mesomycoplasma hyorhinis</i>	0
3	<i>Mycoplasma arginini</i>	0
4	<i>Metamycoplasma orale</i>	0
5	<i>Mycoplasma synoviae</i>	0
6	<i>Spiroplasma citri</i>	0
7	<i>Mycoplasma fermentans</i>	0
8	<i>Mycoplasma gallisepticum</i>	0
9	<i>Mycoplasma pneumoniae</i>	0
10	<i>Metamycoplasma hominis</i>	0
11	<i>Ureaplasma urealyticum</i>	0
12	<i>Mycoplasma genitalium</i>	0
13	<i>Streptococcus pneumoniae</i>	-
14	<i>Lactobacillus crispatus</i>	-
15	<i>Escherichia coli</i>	-

This test includes a highly-specific primer set, which sensitive and specific detection of several different species (see the specificity table Number 1~12). Also, it has been confirmed that there is no cross-reactivity with *Escherichia coli* and Gram-positive bacteria that are closely related to *Mycoplasma* (see the specificity table Number 13~15).

## TROUBLESHOOTING GUIDE

1. No signal increase is observed, even with positive controls	
<ul style="list-style-type: none"> <li>• Incorrect detection channel has been chosen</li> <li>• Pipetting errors</li> <li>• No data acquisition programmed.</li> </ul>	<ul style="list-style-type: none"> <li>• Set Channel settings to FAM and Cy5</li> <li>• Check for correct reaction setup. Repeat the PCR run.</li> <li>• Check the cycle programs</li> </ul>
2. Fluorescence intensity is too low	
<ul style="list-style-type: none"> <li>• Low initial amount of target DNA.</li> </ul>	<ul style="list-style-type: none"> <li>• Increase the amount of sample DNA. Exchange all critical solutions.</li> </ul>
3. Negative control samples are positive	
<ul style="list-style-type: none"> <li>• Carry-over contamination.</li> </ul>	<ul style="list-style-type: none"> <li>• Repeat the complete experiment with fresh aliquots of all reagents.</li> <li>• Always handle samples, kit components, and consumables in accordance with commonly accepted practices to prevent carry-over contamination.</li> <li>• Add positive controls after the sample and negative control reaction vessels have been sealed.</li> </ul>
4. Fluorescence intensity varies	
<ul style="list-style-type: none"> <li>• Insufficient centrifugation of the PCR strips. Resuspended PCR mix is still in the upper part of the vessel.</li> <li>• Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).</li> </ul>	<ul style="list-style-type: none"> <li>• Centrifuge PCR strips.</li> <li>• Always wear gloves when handling the vessels and seal</li> </ul>
5. Baseline drift phenomenon	
<ul style="list-style-type: none"> <li>• Invalid baseline setting applied.</li> </ul>	<ul style="list-style-type: none"> <li>• Settings → Baseline Setting → Apply fluorescence Drift Correction.</li> </ul>

## SYMBOL GLOSSARY

Symbol	Meaning	Symbol	Meaning
<b>REF</b>	Catalogue number		Manufacturer
<b>LOT</b>	Batch code		Do not reuse
	Temperature limit	<b>RUO</b>	Research use only
	Use-by date		Consult instructions for use
	Contents sufficient for <n> tests		Keep away from sunlight

## ORDERING INFORMATION

Product	Cat No.	Package
WizDx™ CrystalMix Mycoplasma	DX1241	96 Test

### Technical Support

[www.wizbiosolution.com](http://www.wizbiosolution.com)  
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