MYCOPLASMA REAL TIME PCR KIT

Cat No: 17R-10-01

DESCRIPTION

SNP Mycoplasma Real Time PCR kit is performed to detect the mycoplasma spp. in biological materials, such as cell cultures.

PRINCIPLE OF THE SYSTEM

During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

MYCOPLASMA TYPES CAN BE DETECTED

M. fermentans	*M. gallisepticum*	*M. ovipneumoniae*
M. hyorhinis	*M. pulmonis*	*M. pirum*
M. arginini	*M. arthritidis*	*M. capricolu*
M. orale	*M. bovis*	Acholeplasma
M. salivarium	*M. pneumoniae*	Spiroplasma
M. hominis	*M. haemofelis*	
M. genitalium	*M. hyopneumoniae*	

SYSTEM CONTENTS

Reagents	20 rxns
• Mycoplasma Master Mix	400 µl
• Positive Control DNA	20 µl

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>3X) should be avoided, as this may reduce the sensitivity of the assay.

PROCEDURE

- Before starting work, mix the mastermixes gently by pipetting
- For each sample, pipet **20 µl mastermix*** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl (~10-100 ng) DNA** into each tube**.**
- Run with the programme shown below.

Master mixes include HotStart Taq DNA Polymerase.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	30 Cycles
60 °C	1 Min.	

Fluorescent dyes are FAM and HEX/JOE

If you use;

- ABI Prism® system, please choose **"none"** as passive reference and quencher.
- Mic qPCR Cycler, please adjust gain settings, **"Green Auto Gain"** to **20** and **"Yellow Auto Gain"** to **10.**

This system can use with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

Roche LightCycler® 480 System

Rotor Gene Q

Mic qPCR Cycler

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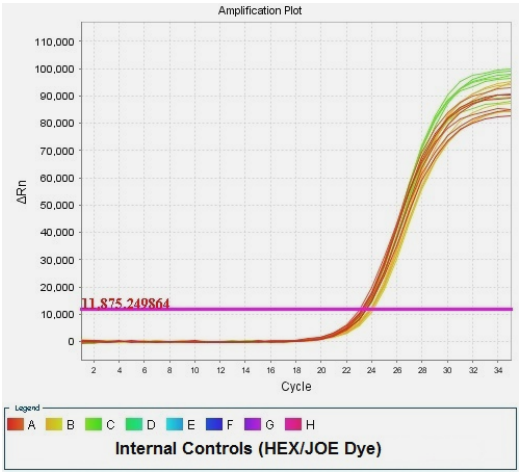
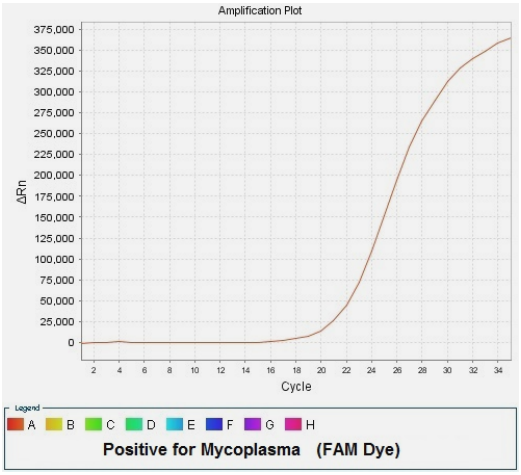
DATA ANALYSIS

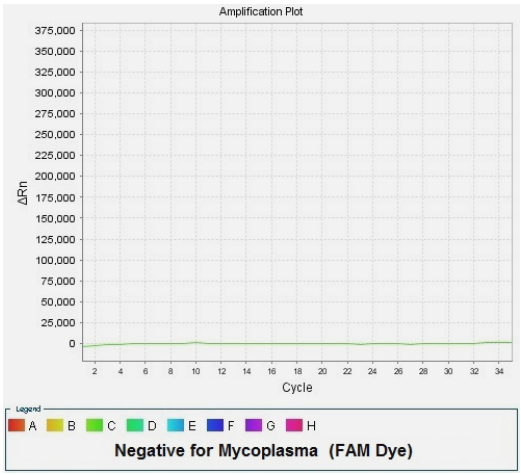
After the run is completed data are analysed using the software with HEX (JOE) and FAM dyes. The below results were studied with ABI 7500.



Internal control amplification plots must be seen in all wells and has been labelled with HEX/JOE dye..



Amplification plots of mycoplasma can be analysed by FAM dye



TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

If plots start late,

Compare positive control and sample. If there is no problem in positive control,

- DNA quality is not good.
- The amount of DNA is not enough.

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.