

TransDetect® Direct qPCR Mycoplasma Detection Kit

Please read the manual carefully before use

Cat. No. FM331

Version No. Version 1.0

Storage: at -18°C or below for two years in dark

Description

This product utilizes the TaqMan probe-based qPCR method to the detection of mycoplasma DNA for qualitative identification of mycoplasma contamination. It offers a broad detection range covering various species within the class *Mollicutes*, including *Mycoplasma spp.*, *Acholeplasma spp.*, and *Spiroplasma spp.* The TransDetect® qPCR Mycoplasma SuperMix II included in the kit contains hot-start Taq DNA polymerase, a qPCR reaction buffer specially optimized for mycoplasma detection, dNTPs, PCR enhancer, and stabilizer. In addition, a dUTP/UDG system is introduced into this reaction mix, which can degrade U-containing ssDNA and dsDNA prior to amplification, eliminating carry-over contamination caused by PCR products. The Myco Primer & Probe Mix II includes primers and probes for amplification of both mycoplasma sequences and the Myco Internal Control Template II. The FAM channel detects mycoplasma-specific amplification, while the ROX channel monitors the amplification of the Myco Internal Control Template II. This kit can be used directly to detect mycoplasma in samples such as cell culture supernatants, cell culture media, and serum.

Features

- High sensitivity, broad mycoplasma species coverage, excellent durability, and high specificity.
- Incorporates a dUTP/UDG anti-contamination system, effectively eliminating carry-over contamination from PCR products and ensuring accurate results.

Kit Contents

Component	FM331-01 (50 rxns)	FM331-02 (100 rxns)
TransDetect® qPCR Mycoplasma SuperMix II	750 µl	2×750 µl
Myco Primer&Probe Mix II	250 µl	500 µl
Myco Internal Control Template II	50 µl	100 µl
Myco Positive Control Template IV	100 µl	200 µl
MycoFree Water	500 µl	1000 µl

qPCR Detection

1. Prepare 28 µl qPCR premix (in reagent preparation area): Equilibrate all components to room temperature. Mix well before use. The following reaction setup corresponds to the volume for a single reaction well.

Component	Volume
TransDetect® qPCR Mycoplasma SuperMix II	15 µl
Myco Primer&Probe Mix II	5 µl
Myco Internal Control Template II	1 µl
MycoFree Water	7 µl

2. Sample loading (in the sample handling area): Add the sample, vortex to mix, and briefly centrifuge.

Sample type	Volume
Positive control	28 µl qPCR premix + 2 µl Myco Positive Control Template IV
Negative control (NTC)	28 µl qPCR premix + 2 µl MycoFree Water
Test sample	28 µl qPCR premix + 2 µl test sample

3. qPCR amplification (in the amplification and analysis area)

Put the PCR reaction tubes into the sample tank of the amplification instrument, set the negative control(NTC), positive



control and test sample in the corresponding order. Select the FAM channel and ROX channel. Set the reaction volume to 30 μ l. Set the reaction program as follows:

Temperature	Time	Cycle
95°C	5 min	1
95°C	5 sec	45
62°C	30 sec (Fluorescence signal acquisition)	

Result Analysis

The amplification results of positive control and negative control (NTC) need to meet the following table:

Sample	FAM channel	ROX channel
Positive control	Ct < 40	Ct < 40
NTC	Ct $\geq$ 40	Ct < 40

Interpretation of results for samples to be tested according to the table below:

FAM channel	ROX channel	Interpretation
Ct < 40	Ct < 40	Mycoplasma positive
Ct < 40	Ct $\geq$ 40	PCR inhibition
Ct $\geq$ 40	Ct < 40	Mycoplasma negative
Ct $\geq$ 40	Ct $\geq$ 40	PCR inhibition

If the ROX signal is inhibited, it should be retest or properly treated for sample to eliminate the inhibitory factor.

Notes

- Thoroughly thaw and mix well before use.
- This product is only used for scientific research. Please read this manual carefully before use.
- Before the experiment, please be familiar with and master the operation methods and precautions of various instruments to be used, and carry out quality control for each experiment.
- Laboratory management must strictly follow the management specifications of PCR gene amplification laboratories. The experimental personnel must undergo professional training. The experimental process is strictly carried out in separate areas. All consumables are only for one-time use. Each stage of the experimental operation uses specialized instruments and equipments. Supplies in different areas and stages cannot be used interchangeably. Sample handling and waste disposal should comply with relevant laws and regulations.

For research use only, not for clinical diagnosis.

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