TransDetect® qPCR Mycoplasma Detection Kit

Please read the datasheet carefully prior to use.

Cat. No. FM321

Version No. Version 1.0

Storage: at -20°C for two years in dark

Description

This product is designed for detecting mycoplasma DNA by TaqMan probe real-time PCR, which is used to qualitatively detect mycoplasma contamination in samples such as culture media, cell cultures, biological products, etc. This kit can detect a variety of mycoplasma, including *Mycoplasma spp*., *Acholeplasma spp*., *Spiroplasma spp*., etc. under the class *Mollicutes*. This kit was validated in accordance with EP 2.6.7 and JP G3 guidelines and requirements for mycoplasma detection.

TransDetect® qPCR Mycoplasma SuperMix in this product contains Taq hot start enzyme, specially optimized qPCR reaction buffer 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 before reverse transcription, eliminating carry-over contamination caused by PCR products. Primer & Probe Mix is used to amplify mycoplasma sequences and Internal Control. FAM channel detection of mycoplasma-specific amplification; The VIC channel detects the amplification of Internal Control. Internal Control can be added at the time of PCR reaction to exclude false-negative results due to PCR inhibitors in the sample; Internal control can also be added at the time of sample extraction to evaluate the extraction effect and rule out false negatives due to improper DNA extraction. This kit is used in conjunction with the *MagicPure*® 32 Mycoplasma DNA Kit (EH401-32) to efficiently extract mycoplasma DNA from samples, and the detection limit can reach 10 CFU/ml.

Features

- In accordance with the requirements of nucleic acid amplification technology (NAT) detection in EP2.6.7 and JP G3, with high sensitivity, wide coverage of mycoplasma species, excellent durability and excellent specificity.
- This product contains dUTP/UDG anti-contamination system, which effectively prevents carry-over contamination of PCR products to ensure accurate data.

Kit Contents

Component	FM321-01
TransDetect® qPCR Mycoplasma SuperMix	800 µl
Primer&Probe Mix	150 µl
Internal Control	1 ml
Positive Control	400 µl
Nuclease-free Water	1 ml

Sample Purification

In order to ensure detection sensitivity, it is recommended to use this kit for mycoplasma detection after DNA extraction. It is recommended to use the *MagicPure*® 32 Mycoplasma DNA Kit (EH401-32) for DNA extraction and follow the instructions. Internal Control can be added during sample extraction to verify whether the extraction is normal and whether the extraction product has PCR inhibitory components. The amount of Internal Control added to each extracted sample is 10 µl, refer to the EH401-32 product manual for details.

qPCR Detection

1. Determine the number of reaction wells

   Number of reaction wells = (number of samples to be tested + number of extracted negative control samples(NCS) + no template control NTC(1) + positive control(1))× replicates

   The sample to be tested is the extracted DNA sample, and the extraction negative control (NCS) sample is the extraction and recovery product of the mycoplasma negative sample.

2. Reagent preparation (in the reagent preparation area)
   Bring and balance the components in the kit to room temperature. And mix well for later use.
3. qPCR reaction solution preparation(in reagent preparation area and sample handling area)
   Prepare the reaction solution according to the following table. The number of reaction systems N = number of reaction wells + 1. Mix the *TransDetect*® qPCR Mycoplasma SuperMix and Primer & Probe Mix in the reagent preparation area, then add Internal Control in the sample handling area. Mix the prepared qPCR reaction solution thoroughly and centrifuge briefly for later use.

Component	Volume
TransDetect® qPCR Mycoplasma SuperMix	8 μl×N
Primer&Probe Mix	1.5 μl×N
Internal Control	0.5 μl×N

*If Internal Control has been added at the time of extraction, Nuclease-free Water should be used instead of Internal Control when preparing qPCR reaction solution.

4. Template preparation for NTC and positive control (in the sample handling area)
   Refer to the methods described below:
   *If Internal Control is added at the time of preparation of qPCR reaction solution, Nuclease-free Water is used as NTC template and Positive Control is used as positive control template;
   *If Internal Control is not added at the time of preparation of qPCR reaction solution, add Internal Control to the NTC template and the positive control template at a ratio of 0.5 μl of sample to 20 μl of sample, specifically:
   Preparation method of NTC template: number of replicate wells × 20 μl Nuclease-free water + number of replicate wells × 0.5 μl Internal Control
   Preparation method of positive control template: number of replicate wells × 20 μl Positive Control + number of replicate wells × 0.5 μl Internal Control
5. Sample Loading (in the sample handling area)
   (1) Add 10 μl of the qPCR reaction solution prepared in the previous step to each PCR tube.
   (2) Add 20 μl of template to the reaction wells of the aliquoted qPCR reaction solution: positive control template, NTC template, extraction negative control (NCS), sample to be tested, refer to the following table.

Positive control	10 μl qPCR reaction solution + 20 μl positive control template
NTC	10 μl qPCR the reaction solution + 20 μl NTC template
Extraction negative control (NCS)	10 μl qPCR reaction solution + 20 μl extraction negative control (NCS) sample
Test sample	10 μl qPCR of reaction solution + sample to be tested

   It is recommended that the above four types of samples should be placed in different zones during the design and layout of the reaction wells to avoid cross contamination and inaccurate test results. After capping the tube or sealing it with an optical film, gently vortex to mix well, and
   centrifuge to make all the liquid attach to the bottom of the tube.
6. 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 VIC channel. Set the reaction volume to 30 μl. Set the reaction program as follows:

Temperature	Reaction Time	Cycle
95℃	5 min	1
95℃	5 sec	45
62℃	30 sec(Signal Collection)	

Result Analysis

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

Sample	FAM channel	VIC channel
Positive control	Ct＜40	Ct＜40
NTC	Ct≥40	Ct＜40

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

FAM channel	VIC channel	Interpretation
Ct＜40	Ct＜40	Mycoplasma positive
Ct＜40	Ct≥40	PCR inhibition
Ct≥40	Ct＜40	Mycoplasma negative
Ct≥40	Ct≥40	PCR inhibition

If the VIC 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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Service telephone +86-10-57815020
Service email complaints@transgen.com