MycAway™ Mycoplasma Real-time qPCR Detection Kit

Product description

MycAway™ Mycoplasma Real-time qPCR Detection Kit is a product which can qualitative detect the mycoplasma contamination in the raw materials, cell bank, virus seeds, virual or cell harvesting solution and cells used in clinical treatment, etc. The kit uses the Taqman fluorescent probe (which included FAM and VIC) and the Multiple polymerase chain reaction (PCR) tools to detect the target and internal control separately. It covered over 100 species of the Mollicutes DNA, the specificity, detection limit and robustness of this kit are validated according to EP2.6.7 which with high sensitivity, specificity, efficiency and safety. The detection limit is equals to and below 10 CFU/mL.

This product can be used in combination with MolPure® Magnetic Residual DNA Sample Preparation Kit (Cat#18461) which using the manual extracted method for the nucleic acid extraction. The nucleic acid in samples can also be automatically extracted by Auto-Pure 32A automatic nucleic acid extractor (Cat#80501) and MolPure® Mag32 Residual DNA Sample Preparation Kit FA(Cat#18462)(Please note, the kits which included Cat#18461 and Cat#40618 are full validated, please contact our technical support for detailed validation information). After the samples are pre-treated to remove the interference impurities and obtain purified nucleic acid, then a qPCR reaction perform by the Real Time PCR amplifier and the fluorescence signal of the probe will be collected and analyzed.

Specifications

Cat.No.	40618ES25 / 40618ES60
Size	25 T / 100 T

Components

Components No.	Name	40618ES25	40618ES60
40618-A	4×MyqPCR Reaction Buffer	250 μL	1 mL
40618-B	MyPrimer & Probe MIX	25 μL	100 μL
40618-C*	Internal Control (IC)	25 μL	100 μL
40618-D**	Positive Control (PC)	500 μL	2 mL
40618-E***	DNA Dilution buffer	1 mL	4×1 mL
40618-F****	Ultrapure water	500 μL	2×1 mL

*IC: Internal control;

**PCS: Positive control solution，the concentration is 1,000 copies/μL.

***DNA Dilution buffer: used for IC dilution and the template of NTC and NCS.

****Ultrapure water: used for the preparation of qPCR Mix。

Storage

This product should be stored at -25~-15℃ for 1 year.

*Upon receipt of the kit, please check whether all components are complete and immediately store them in -25~-15℃ condition if not perform the assay immediately. Please note 40618-B should be stored away from light.

Instructions

1. Preparations before experiment

1）Prepare the required reagents and materials which will be used in the experiment.

2）Confirm the suitability of the qPCR instrument

This kit can be used on the types of qPCR instrument as below:

a. Bio-Rad: CFX96

b. Thermo Scientific: 7500 Real-Time PCR System；QuantStudio™ 5；

2. Experiment method

1）DNA Extraction

We recommend to use ‘Magnetic Residual DNA Sample Preparation Kit’ (Cat#18461ES for manual extraction and Cat#18462ES for auto extraction) for the DNA extraction, you can visit ‘http:/www.yeasenbiotech.com’ for the detailed information and the purchasing.

The kit (Cat#40618ES) contains an internal control (IC). If add the IC to the samples prior to DNA extraction, it can verify the complete process (included DNA extraction and qPCR reaction). If add the IC to the qPCR master mix directly, the IC will acts as a qPCR control only.

2）qPCR Mix preparation

a. According to the sample amount which included Positive control (PCS), No template control (NTC), Negative control solution (NCS) and Test sample (TS) to calculate the number of reactions. Prepare 2 reactions in parallel for each sample in generally.

*PCS：Positive control solution；NTC：No template control；NCS：Negative control solution；TS：Test sample. There is no need to perform the sample extraction for PCS and NTC, but NCS and TS are needed.

Reaction wells（M1）=（1×NCS＋N×TS）×2

Reaction wells（M2）=（1×PCS＋1×NTC）×2

Reaction wells（M3）=（1×PCS＋1×NTC+ N×TS）×2

b. Pre-thaw the required amount reagents on ice according to the experiment design and Tables below.

c. Calculate the amount of qPCR Mix according to the Number of reactions. Please note, if the kit will be used for GMP activities such as product release, we recommended used Table 1 and 2 for the preparation; If the kit will just used for research and there is no need to add IC before the extraction after the evaluation, then follow Table 3 for the preparation. Please note that not all M1, M2 and M3 are needed to be prepare.

Component	Volume（1×40μL Reactions）	Volume（M1×40μL）
4× MyqPCR Reaction Buffer	10 μL	(M1+2) ×10 μL
MyPrimer & Probe MIX	1 μL	(M1+2) ×1 μL
ROX	0.8 μL /0 μL**	(M1+2) ×0.8 μL /0 μL
Purified water	Up to 20 μL	Up to (M1+2) ×20 μL
Total	20 μL	(M1+2) ×20 μL

Table 1 qPCR Mix system for M1

*The configuration system in Table 1 is based on the premise that IC is added before extraction for both NCS and TS, so it is not necessary to add IC when qPCR Mix preparation. IC adding method before extraction: Firstly, dilute IC by 20 times with DNA diluent, and add 1μL diluted IC into each 100μL test sample for the further extraction.

**This kit does not contain ROX Reference Dye. If ROX reference dye is needed for the Real Time PCR amplifiers that you are currently using, 50×ROX Reference Dye (Cat#10200ES) is recommended for use. In this case, the added volume is 0.8μL, as shown in Table 1. If using other brands of ROX products, please refer to their instructions for ROX addition. If no ROX reference dye is required, the added volume is 0 μL.

Component	Volume（1×40μL Reactions）	Volume（M2×40μL）
4× MyqPCR Reaction Buffer	10 μL	(M2+2) ×10 μL
MyPrimer & Probe MIX	1 μL	(M2+2) ×1 μL
Internal Control（IC）	1 μL*	(M2+2) ×1 μL /0 μL
ROX	0.8 μL /0 μL**	(M2+2) ×0.8 μL /0 μL
Purified water	Up to 20 μL	Up to (M2+2) ×20 μL
Total	20 μL	(M2+2) ×20 μL

Table 2 qPCR Mix system for M2

*The configuration system in Table 2 is based on the premise that IC is not added in PCS and NTC before extraction, so IC needs to be added during qPCR Mix preparation. IC adding method after extraction: Dilute IC by 100 times with DNA diluent and add 1μL diluted IC into each qPCR Mix system.

**This kit does not contain ROX Reference Dye. If ROX reference dye is needed for the Real Time PCR amplifiers that you are currently using, 50×ROX Reference Dye (Cat#10200ES) is recommended for use. In this case, the added volume is 0.8μL, as shown in Table 1. If using other brands of ROX products, please refer to their instructions for ROX addition. If no ROX reference dye is required, the added volume is 0 μL.

Component	Volume（1×40μL Reactions）	Volume（M3×40μL）
4× MyqPCR Reaction Buffer	10 μL	(M3+2) ×10 μL
MyPrimer & Probe MIX	1 μL	(M3+2) ×1 μL
Internal Control（IC）	1 μL*	(M3+2) ×1 μL /0 μL
ROX	0.8 μL /0 μL**	(M3+2) ×0.8 μL /0 μL
Purified water	Up to 20 μL	Up to (M3+2) ×20 μL
Total	20 μL	(M3+2) ×20 μL

Table 3 qPCR Mix system for M3

*The configuration system in Table 3 is based on the premise that IC is not added to samples before extraction, so IC needs to be added during qPCR Mix preparation. IC adding method after extraction: Dilute IC by 100 times with DNA diluent and add 1μL into each qPCR Mix system.

**This kit does not contain ROX Reference Dye. If ROX reference dye is needed for the Real Time PCR amplifiers that you are currently using, 50×ROX Reference Dye (Cat#10200ES) is recommended for use. In this case, the added volume is 0.8μL, as shown in Table 1. If using other brands of ROX products, please refer to their instructions for ROX addition. If no ROX reference dye is required, the added volume is 0 μL.

3）Templates adding

a. Mix the qPCR Mix with sufficient shaking, centrifuge at low speed and collect the residual liquid from the cap to the bottom of the tube.

b. Add 20 μL qPCR Mix to each reaction tube/well. Please note adding the corresponding qPCR Mix into each sample tubes and avoid adding errors.

c. Add Templates to the tube/wells which contained the qPCR Mix. See Table 4 for the templates adding.

Test Samples	In each tube or well…
TS	20 μL qPCR Mix+20 μL Samples after extraction
NTC	20 μL qPCR Mix+20 μL DNA Dilution buffer
NCS*	20 μL qPCR Mix+20 μL Negative sample after extraction*
PCS	20 μL qPCR Mix +20 μL Positive control

Table 4 Templates adding

*We recommend to use DNA Dilution buffer (40618-E) as the template of NCS for the DNA extraction.

**The total reaction volume in each tube/well is 40 μL.

***Cover the tube lid or the plate film. To avoid affecting the fluorescence signal reading, please take care not to mark the tube lid or film or even rub the film repeatedly with a scraper.

****Centrifuge the reaction tube or plate briefly at low speed after templates adding. After sufficient shaking and mixing, repeat centrifuge at low speed to collect the liquid from the lid or wall to the bottom. Avoid bubbles when operation. The baseline will be impacted if the mix is not mixed well, so this step is very important to a good experiment result.

4) qPCR programs setting

a. Program file Settings

Example (7500 Real-Time PCR System instrument and Real-Time PCR Software v2.4):

Instrument type：7500 (96 Wells)

Experiment type：Quantitation-Standard Curve；

Chemistry：Taqman® Reagents

Ramp Speed：Standard (~2 hours to complete a run)

b. Target channel Settings

In "Define Targets and Samples" of "Plate Setup", create a Target 1 channel (FAM), select FAM as the reporting fluorescence group and MGB or none as the quenching fluorescence group. Create a Target 2 channel (VIC), select the reporting fluorescence group as VIC and the quenching fluorescence group as none. In "Assign Targets and Samples" of "Plate Setup", if no additional ROX dye is added, select "none"; If an additional ROX is added, select ROX.

c. Standard amplification program Settings

S/N	Reaction Stage	Temperature	Time	Cycle(s)
1	Initial denaturation	95°C	5 min	1
2	Denaturation	95°C	15 sec	45
3	Annealing/Extension (fluorescence signal collection)	62°C	30 sec	

Table 5 Standard amplification program Settings

d. Baseline and threshold setting:

Principle of baseline adjustment: use the automatic baseline generally. If need to adjust in manual, choose the cycle before the exponential growth period as the start cycle, and avoid the fluctuation zone of initial fluorescence collection. Choose the cycle which is 1-2 cycles before the Ct of the earliest exponential amplification sample as the end point.

Principle of threshold adjustment: use the automatic threshold generally. If need to adjust in manual, the threshold should be set higher than the Negative sample or the baseline noise, it’ s generally set the threshold in the late stage of the exponential amplification, relative independent and suitable threshold is needed for each tunnel.

5）Result Analysis

a. Result judgement for PCS, NTC and NCS:

If IC added: the specification of each control samples should be satisfied in Table 6:

Control sample	FAM Signal	VIC Signal
PCS	Ct < 40, and has obvious amplification curve	Ct < 40 and has obvious amplification curve
NTC	Ct ⩾ 40 or no obvious amplification curve	Ct < 40 and has obvious amplification curve
NCS	Ct ⩾ 40 or no obvious amplification curve	Ct < 40 and has obvious amplification curve

Table 6 Result judgement of PCS, NTC and NCS

If IC not added: each quality control sample shall meet the specification of FAM signal column in Table 6, and no need to analyze VIC channel.

b. Result judgement for TS

Prerequisite: It is necessary to determine whether PCS, NTC and NCS passed the specification in Table 6 before TS results analysis. If passed, then can proceed to the next step. If not passed, the TS results may not be reliable, and the reason needs to be investigated.

If IC added: find the corresponding results judgement according to the result information of FAM and VIC in Table 7:

FAM Signal	VIC Signal	Result judgement
Ct<40 and has obvious amplification curve	Ct<40 and has obvious amplification curve	Positive
	Ct⩾40 or no obvious amplification curve	An inhibition is existed, the experiment need to be repeated
Ct⩾40 or no obvious amplification curve	Ct<40 and has obvious amplification curve	Negative
	Ct⩾40 or no obvious amplification curve	An inhibition is existed, the experiment need to be repeated

Table 7: Result judgement of TS (IC added)

*If there is inhibition for VIC signal, treatment is needed to eliminate the inhibitors or repeat the test.

If IC not added: find the corresponding results judgement according to the result information of FAM in Table 8 , and it is no need to analyze the VIC signal.

FAM Signal	Result judgement
Ct<40 and has obvious amplification curve	Positive
Ct⩾40 or no obvious amplification curve	Negative

Table 8: Result judgement of TS (IC not added)

Notes

1. Please read this manual carefully before using this kit. The experiment should be conducted in a standardized manner, including sample handling, preparation of reaction system and sample addition.
2. Keep operations of sample adding and reagents preparing on ice if possible.
3. Vortex and mix well for each reagents before use.

4. Please operate with lab coats and disposable gloves，for your safety.
5. This product is for research use only.