Ver. HB221130

MycAway™ Mycoplasma Real-time qPCR Detection Kit

Product description

MycAway™ Mycoplasma Real-time qPCR Detection Kit is a product which can accurately detect the mycoplasma and to verify if there is mycoplasma contamination in the cell bank, virus seeds, biological products and cells used in clinical treatment, etc. The kit uses the Taqman fluorescent probe and the polymerase chain reaction (PCR) tools to qualitative detect the mycoplasma DNA in the test samples and can cover the mycoplasma DNA over 100 species. The kit validation is performed in strict accordance with the mycoplasma testing requirements in EP2.6.7 and JPXVII and with high sensitives, specificity, efficiency and safety.

The kit contains an internal control (IC), which can be used to determine if there is inhibitor in the sample to the amplification reaction, thus preventing false negative results; The IC can also be added during the sample extraction step to evaluate the extraction effect. This kit is used in conjunction with the Mycoplasma DNA Extraction and Purification Kit to efficiently extract mycoplasma DNA with a detection limit of 10 CFU/mL and a maximum sensitivity of 1 CFU/mL.

Components

Components No.	Name	40618ES25 (25 T)	40618ES60 (100 T)
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

[Notes]: The conc. of the positive control (PC) is 1,000 copies/µL.

Shipping and Storage

All the components are shipped with dry ice and can be stored at -15°C~ -25°C for one year.

Instructions

1.DNA extraction

1.1 Prepare the DNA template for the PCR reactions using the MolPure™ Magnetic Residual DNA Sample Preparation Kit.

2. qPCR reaction system

2.1 According to the sample amount which included positive control (PC), no template control (NTC), negative control sample (NCS) and unknow samples (UNK) to calculate the number of reactions, prepare 2 reactions in parallel for each sample in generally.

Number of reactions = (1×PC + 1×NTC + 1×NCS + N×UNK) ×2

2.2 Pre-thaw the required amount reagents on ice.

2.3 Calculate the amount of qPCR Mix according to the Number of reactions, see the table below for the components amount.

Table 1 qPCR Mix System

Component	40 μL reaction	N×40 μL reaction
4× MyqPCR Reaction Buffer (1× in the final volume)	10 μL	(N+2) × 10 μL
MyPrimer&Probe MIX (0.4 μM in the final volume)	1 μL	(N+2) × 1 μL
IC	1 μL /0 μL*	(N+2) × 1 μL /0 μL
Ultrapure water	Up to 20 μL	Up to (N+2) × 20 μL
Total	20 μL	(N+2) × 20 μL

[Notes]:2.3.1 To ensure the stability of the experiment, store the components 40618-A and 40618-B at -20°C.

2.3.2 To reduce the number of freeze-thaw and avoid the cross contamination, it is recommended to sub-package 40618-C and 40618-D when first used, then stored at -80°C.

2.3.3 Add DNA template (included PC, NTC, NCS and UNKs) to each tube/well separately, not as part of qPCR Mix System.

*1 μL/0 μL represents whether IC is added. It is recommended to add IC when using the kit, which can be better confirm whether the system is configured correctly. If not, please refer to the result analysis guidance for result judgment.

3. Templates adding

3.1 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.

3.2 Add 20 μL qPCR Mix to each reaction tube/well.

3.3 Add Templates to the tube/wells which had contained the qPCR Mix. See Table 2 for the templates adding.

Table 2: Templates adding

Test Sample	In each tube or well…
UNK	20 μL qPCR Mix + 20 μL Unknow samples
NTC	20 μL qPCR Mix + 20 μL ddH_2O
NCS	20 μL qPCR Mix + 20 μL DNA Elution Buffer
PC	20 μL qPCR Mix + 20 μL Positive control

[Note]: The total reaction volume in each tube/well is 40 μL.

3.4 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.

3.5 Briefly centrifuge the reaction tube or plate at low speed After sufficient shaking and mixing, repeat centrifuge to collect the liquid from the lid or wall to the bottom. Avoid bubbles when operation.

4. Cycling Protocol

4.1 Generate Target tunnel probe:

Add Targets 1 (FAM), and set the Reporter as FAM, Quencher as none.

Add Targets 2 (VIC), and set the Reporter as VIC, Quencher as none.

Add the Passive Reference as ROX (Just for reference, this kit doesn’ t contain ROX).

4.2 Standard amplification procedures:

S/N	Reaction Stage	Temperature	Time	Cycle(s)
1	Initial denaturation	95°C	5 min	1
2	Amplification	95°C	15 sec	45
		62°C*	30 sec	

[Note]: * Fluorescence signal.

5. Result Analysis

5.1 Guidance for PC, NTC and NCS

Control sample	FAM Signal	VIC Signal
PC	C_t<40 and has obvious amplification curve.	Ct<40 and has obvious amplification curve.
NTC	C_t≥40 or no obvious peak.	C_t<40 and has obvious amplification curve (Add IC in Mix).
		C_t≥40 or no obvious peak (No IC added in Mix).
NCS	C_t≥35 or no obvious peak.	C_t<40 and has obvious amplification curve (Add IC in Mix).
		C_t≥40 or no obvious peak (No IC added in Mix).

5.2 Guidance for UNK:

FAM Signal	VIC Signal	Result Judgment
C_t<40 and has obvious amplification curve	Ct<40 and has obvious amplification curve	Positive
	Ct≥40 or no obvious peak	Inhibition
C_t≥40 or no obvious peak	C<40 and has obvious amplification curve	Negative
	Ct≥40 or no obvious peak	Inhibition

[Note]: If there is inhibition for VIC signal, treatment is needed to eliminate the inhibitors or repeat the test.

6. Applicable Instrument

PRISM® 7500 Real-Time PCR System, QuantStudio™ 5 (ABI), CFX96 (Bio-Rad)

Notes

1. Please read this manual carefully before using this kit. The experiment should be performed in a standardized manner, including sample processing reaction system preparation and sample addition.
2. It is better to operate on ice during the sample addition and solution preparation.
3. Ensure to vortex each component sufficiently before use and centrifuge at low-speed.
4. For your safety and health, please wear lab coats and disposable gloves for operation.
5. This product is for research use ONLY!