Cat.: BP-QN22-50

Mycoplasma DNA Detection Kit (qPCR) V01

Product Name
Mycoplasma DNA Detection Kit (qPCR)

Package Specifications
50 Reactions

Expected Use
This reagent kit is used for qualitative detection of whether there is contamination of Mycoplasma in various biological materials such as cell cultures, experimental animal secretions, and animal sera.

Product Description
This reagent kit uses the PCR-fluorescence probe method to design specific primers and probes for the conserved regions of Mycoplasma 16S rRNA sequence, which can specifically amplify Mycoplasma DNA with the characteristics of easy operation, rapid detection, strong specificity and high sensitivity.

An internal control (IC) can be added during sample extraction to evaluate the extraction efficiency or during PCR amplification to monitor whether there is inhibition in the amplification reaction, thus preventing false negative results.

This reagent kit contains UDG enzyme to prevent contamination of amplified products.

When used together with Biori's nucleic acid extraction or purification reagent kits, this reagent kit efficiently extracts Mycoplasma DNA from samples with a detection limit of up to 5 CFU/mL.

Components
The kit contains the following components:

Components	Specification (50 Reactions)
My qPCR MIX	1.0 mL × 1 tube
My positive control	1.5 mL × 2 tubes
My negative control	1.5 mL × 2 tubes
My internal control *	1.0 mL × 1 tube
instructions	1 copy

Note: The components of different batches of reagent kits cannot be interchanged.

Reagents needed for experimental operations but not provided in the kit: nucleic acid extraction or purification kit.

Storage Conditions and Shelf Life
1. Store in the dark at ≤-20℃ with a validity period of 24 months.
2. Avoid repeated freeze-thaw cycles for the kit; the number of freeze-thaw cycles should not exceed 10.
3. Product batch number and expiration date: Refer to the product outer packaging.

Applicable Devices
Including but not limited to the following models: SLAN-96P and SLAN-96S fully automated medical PCR analysis systems; ABI7500 and ABI QuantStudio™ 5 real-time fluorescent quantitative PCR systems; Roche LightCycler 480 fluorescent quantitative PCR system; and Perlor CFX96 quantitative PCR system.

Related Equipment
Clean benches or biosafety cabinets, handheld centrifuges, vortex mixers, real-time PCR instruments, and pipettes of various specifications.

Protocol
It is recommended to conduct experimental operations in a clean bench or biosafety cabinet whenever possible. Prior to use, expose the work surface, pipettes, and centrifuge tube racks to UV light for 30 minutes, then spray with 75% ethanol and wipe dry.

Take out Mycoplasma qPCR MIX, Mycoplasma internal control, Mycoplasma negative control, and Mycoplasma positive control from the reagent kit, allow them to melt at room temperature, shake well and centrifuge briefly for later use.

1. Reagent preparation

Calculate the required number of reaction wells N based on the number of samples to be tested, usually with 2 replicate wells per sample. Take N PCR reaction tubes and add 20μL of Mycoplasma qPCR MIX to each tube.

N = (number of samples + 1 negative control + 1 positive control + 1 no-template control) x2

2. Nucleic acid extraction and purification

Extract and purify nucleic acids from the negative control, and test samples using a nucleic acid extraction or purification kit following the instructions provided by Biori. Add 10μL internal standard to each sample with a sample volume of 200μL.

3. Amplification and detection:

3.1 After adding 20μL/ tube of Mycoplasma qPCR MIX according to the table below into each reaction tube separately, add negative controls, positive controls, as well as processed test nucleic acid solution.

Components	Single well volume (μL)
My qPCR MIX	20
My DNA	20
Total Volume	40

*If the internal control feature of the kit is not used, there is no need to add an internal control during extraction. The program setup should exclude the VIC channel, and the VIC results can be disregarded during data analysis.

*If the internal control was not added during sample extraction but is included during amplification, add 1 μL of internal control to each reaction mix. When preparing the qPCR mix, dispense 21 μL per reaction, resulting in a total PCR reaction volume of 41 μL per reaction.

3.2 The layout of the plate can be referenced in the table below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC					S1	S1					PC
B	NTC					S2	S2					PC
C						S3	S3					
D						S4	S4					
E						S5	S5					
F						S6	S6					
G	NCS					S7	S7					
H	NCS					S8	S8					

This example demonstrates the detections of 1 template-free control NTC, 1 negative control sample NCS, 1 positive control PC, and 8 test samples, with each test performed in duplicate wells.

*For actual testing, adjust the layout according to sample quantity by referring to this example.

3.3 Cover the reaction tube with a lid after preparing the reaction solution, mix well and centrifuge momentarily before transferring it onto a fluorescence PCR instrument.

3.4 Place the reaction tubes in the sample slot of the fluorescence PCR machine and run the following program on it.

Step	Condition	Cycles
UDG treatment	50℃: 2 minutes	1
Pre-denaturation	95℃: 3 minutes	1
PCR amplification	95℃: 10 seconds, 60℃ (fluorescence collection): 30 seconds	45
cooling	25℃*: 10 seconds	1

Select FAM and VIC as the fluorescence channels, where FAM is the target gene and VIC is the internal control gene. For ABI series instruments, choose ROX as the reference fluorescence.

If the instrument does not support 25℃, set to 37℃.

4.Interpretation of Results

4.1 Threshold Line Setting

The threshold line is adjusted based on instrument noise conditions, with the threshold line set beyond the background signal fluctuation position. For manual adjustment, it should be precisely positioned above the highest point of the negative control amplification curve. Threshold line setting should be based on laboratory validation data, with the requirement to meet the detection limit as the benchmark.

4.2 Experimental Establishment Conditions

Quality control sample	FAM	VIC
NTC	Double well Ct ≥ 40 or no significant peak in the amplification curve	/
NCS	Double well Ct ≥ 40 or no significant peak in the amplification curve	Double well Ct value <35 with effective "S" -shaped amplification
PC	Double well Ct value <35 with effective "S" -shaped amplification	/

Quality control standards should be based on laboratory validation data, with the detection limit requirement as the benchmark.

5.3 Interpretation of Results

FAM	VIC	Result Evaluation
Among the two duplicate wells, more than one well showed Ct values <40 and effective "S" -shaped amplification.	Double well Ct value <35 with effective "S" -shaped amplification	Positive
	Double well Ct ≥ 35 or no significant peak in the amplification curve	Positive, with inhibition
Double well Ct ≥ 40 or no significant peak in the amplification curve	Double well Ct value <35 with effective "S" -shaped amplification	Negative
	Double well Ct ≥ 35 or no significant peak in the amplification curve	The result cannot be determined, with inhibition observed

If the VIC channel is suppressed, retesting is required or the inhibitory factors should be identified and eliminated.

Notes

1. The kit should be stored at-20℃ or below.
2. Before conducting the experiment, please read the kit instructions carefully and follow the operational procedures strictly.Precise control of time and reagent volume during operation will yield optimal results.
3. Ensure that consumables used for nucleic acid extraction are clean and DNase/RNase.free. The extraction process should be completed as quickly as possible, followed by proceeding to the next experimental step or cryopreservation.
4. Do not use expired components or mix components from different batches.
5. Freezing storage reagents should be completely thawed at room temperature before use, followed by instantaneous centrifugation to ensure complete sedimentation of the liquid at the tube bottom. Avoid repeated freeze-thaw cycles to prevent degradation of reagent performance.
6. Use new disposable polyethylene gloves to seal the reaction tube lid to avoid contact with the reaction tube using bare hands or used gloves. During testing, employ disposable powder-free latex gloves without fluorescent substances.
7. The laboratory shall strictly implement zoned management in accordance with relevant regulations, conducting genetic testing in the sequence of solution preparation area → template extraction area → amplification area → analysis area. Strict requirements must be enforced regarding personnel, equipment, reagents, and airflow direction within each zone.
8. Samples and positive controls should be promptly sealed after use to avoid contamination between components and aerosols, which may lead to false positives.
9. Amplification products must not be opened, and experimental waste should be collected promptly and disposed of harmlessly away from the PCR laboratory.
10. If the target in the sample is strongly positive, competitive inhibition within the system may affect the detection of the internal standard.

Disclaimer

This product is for research use only, and the liability of our company for this product is limited to the value of the product itself.

Corporate Information

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