SHENTEK

MycoSHENTEK®
Mycoplasma Real-Time PCR Detection

Introduction

The MycoSHENTEK® Mycoplasma Extraction and Detection Kits are designed for rapid and sensitive mycoplasma detection via validated NAT (nucleic acid amplification tests) techniques as an alternative to the broth/agar culture method and/or the indicator cell culture method. The PCR assay is suitable for the detection of Mycoplasma contamination in cell banks, viral seed stocks, raw materials, cell culture, unprocessed bulk samples, and clinical therapeutic products, etc.

- MycoSHENTEK® mycoplasma DNA extraction and detection kits (2G) improve DNA extraction efficiency in complex matrix, for example, biological samples with 5% human albumin or 10^7 cells/mL, etc. The sensitivity meets 10 CFU/mL detection limit, with optimal performance from DNA extraction effectiveness and PCR amplification efficiency.

MycoSHENTEK® Mycoplasma DNA Detection Kit (2G) (qPCR Method)

- Enabling detection of more than 200 species of Mycoplasma, Spiroplasma and Acholeplasma;
- Multiplex PCR design (with internal quality control) for rapid and effective assay detection;
- Highly sensitive and specific detection of Mycoplasma DNA in different and complex matrices;
- Assay performance validated by third-party laboratories and in compliance with pharmacopoeia regulations;

MycoSHENTEK® Mycoplasma DNA Extraction Kit (2G)

- Quantitative and reproducible recoveries for complex matrices, enabling the high sensitivity of 10cfu/mL;
- Compatible with the rHCDpurify® Sample Purification System for automated nucleic acid extraction;

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- MycoSHENTEk® Mycoplasma DNA detection assay has been fully validated with reference to the Chinese Pharmacopoeia ‹9201›, the European Pharmacopoeia Chapter 2.6.7 and the Japanese Pharmacopoeia XVII. With a validated sensitivity of 10 CFU/mL, the 2G (2nd generation) kit is capable of detecting a wide variety of Mycoplasma species in complex matrices to replace compendial methods. (The NAT test system must be shown to detect 10 CFU/mL as an alternative to the culture method, or to detect 100 CFU/mL as an alternative to the indicator cell culture method.)

Product Number	Product Name	Quantity
1509840	MycoSHENTEK® Mycoplasma DNA Extraction Kit (2G)	50 Extractions
1509841	MycoSHENTEK® Mycoplasma DNA Detection Kit (2G)	50 Reactions

SHENTEK® is authorized by microbial culture collection centers in China (e.g. CMCC) and abroad (e.g. ATCC) to provide below of Mycoplasma standards:

1) MycoSHENTEK® Mycoplasma Sensitivity Standard:

Each tube of sensitivity standard contains 10 CFU or 100 CFU of inactivated mycoplasma, which can be used safely and reliably in the NAT methods. The colony forming unit (CFU) determined by the culture method and the genomic copy (GC) number by dPCR quantitation, are provided in the Certificate of Analysis for each lot. It cannot be used for culture method due to mycoplasma inactivation. The standard solution or the matrix spike are intended for validating sensitivity, specificity, robustness.

Product Number	Product Name	Quantity
1501501	MycoSHENTEK® *Mycoplasma orale* Sensitivity Standard (10 CFU)	5 tubes, 10 CFU/tube
1501503	MycoSHENTEK® *Mycoplasma pneumoniae* Sensitivity Standard (10 CFU)	5 tubes, 10 CFU/tube
1501505	MycoSHENTEK® *Mycoplasma hyorhinis* Sensitivity Standard (10 CFU)	5 tubes, 10 CFU/tube

2) MycoSHENTEK® Mycoplasma gDNA standard:

Each Mycoplasma gDNA standard contain genomic DNA extracted from defined species with GC (Genome copy) calibrated at 1×10^8 copies/μL concentration. They can be used safely and reliably as quantitative controls. The desired concentration is obtained by serial dilution with DNA dilution buffer. They are intended for quantitation, calibration, assessment of assay sensitivity, specificity and robustness.

Product Number	Product Name	Quantity
1502550	MycoSHENTEK® *Mycoplasma orale* gDNA Standard (1×10^8 copies/μL)	1 tube, 100 μL/tube
1502551	MycoSHENTEK® *Mycoplasma pneumoniae* gDNA Standard (1×10^8 copies/μL)	1 tube, 100 μL/tube
1502552	MycoSHENTEK® *Mycoplasma hyorhinis* gDNA Standard (1×10^8 copies/μL)	1 tube, 100 μL/tube

Mycoplasma Test Service

- NAT method: PCR-based method validation, sample suitability test and PCR assay for lot release and in-process test as microbial risk mitigation strategy.
- Culture methods: Culture-based method validation and sample testing according to the compendial guidelines (the culture method & indicator cell culture method) with quality control strain preparation in P2 biological laboratory.
- Sample pre-treatment: Sample lysate preparation, especially pre-treatment of complex matrices (e.g. high-density cell suspensions, blood samples, etc.) for DNA extraction and spike recovery validation.

Mycoplasma Assay Validation

Characteristics		Acceptance Criteria
Inclusivity		Through intensive bioinformatics analysis, optimized multiplex primer design and reaction condition, the system allows genomic DNA detection of more than 200 species of Mycoplasma, Spiroplasma and Acholeplasma, without cross-detection of related bacterial species. The samples spiked at LOD level of each reference strain were detectable at least in 95% of test runs for over 10 species, also for control plasmids containing Mycoplasma DNA.
Detection Limit	Strain detection limit	10 CFU/mL (Positive cut-off: 95%)
	Genomic copy number sensitivity	1 genome copy/μL (Positive cut-off: 95%)
Specificity	Sample matrix effect	Consistent performance across different sample matrices (e.g. culture media, serum, cryopreservation solution, mycoplasma media, etc.), and no matrix-induced effect
	Cross-reactivity	No cross-reactivity with nearly 30 types of production cells, engineered bacteria and fungi
Robustness	Matrix robustness	All spiked samples in high-density (>1 x 10^6 cells/mL) Vero/293T/CHO cell culture gave positive results at the level of detection limit (LOD = 10CFU/mL).
		All spiked samples in different complex matrices (e.g. 5% human albumin, 10^7 cells/mL Vero/293T/CHO cell culture) gave positive results at the level of detection limit (LOD = 10CFU/mL), using 2G kit.
	Freeze-thaw stability	Stable assay performance for at least 5 freeze-thaw cycles
	Instrument suitability	Good consistency of assay performance on different qPCR instruments (e.g. SHENTEK-96S, ThermoFisher ABI 7500, Bio-Rad CFX-96, Roche LightCycler 480, etc.)

MycoSHENTEK® Detection Assays for Different Mycoplasma Species

Table 1. MycoSHENTEK® Sensitivity/Detection Level at 10 & 100 CFU/mL
For Different Mycoplasma Species

Matrix	10CFU/mL	100CFU/mL	Matrix	10CFU/mL	100CFU/mL
Mycoplasma synoviae	24/24	24/24	Mycoplasma hyorhinis	24/24	24/24
Mycoplasma arginine	24/24	24/24	Acholeplasma laidlawii	24/24	24/24
Mycoplasma gallisepticam	24/24	24/24	Mycoplasma fermentans	24/24	24/24
Spiroplasma citri	24/24	24/24	Mycoplasma hominis	24/24	24/24
Mycoplasma orale	24/24	24/24	Mycoplasma pneumoniae	24/24	24/24

Note: 24/24=24 out of 24 test results positive

Table 2. MycoSHENTEK® Sensitivity/Detection Level at 10 & 100 CFU/mL in Complex Matrices (2G kit)
For Different Mycoplasma Species

Matrix		Strain					
		Mycoplasma orale		Mycoplasma hyorhinis		Mycoplasma pneumoniae	
		10CFU/mL	100CFU/mL	10CFU/mL	100CFU/mL	10CFU/mL	100CFU/mL
5% human albumin		24/24	24/24	24/24	24/24	24/24	24/24
10^6 cells/mL	Vero	24/24	24/24	24/24	24/24	24/24	24/24
	293T	24/24	24/24	24/24	24/24	24/24	24/24
	CHO	24/24	24/24	24/24	24/24	24/24	24/24
10^7 cells/mL	Vero	24/24	24/24	24/24	24/24	23/24	24/24
	293T	24/24	24/24	24/24	24/24	23/24	24/24
	CHO	24/24	24/24	24/24	24/24	23/24	24/24

Note: 24/24=24 out of 24 test results positive

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