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SHENTEK

MycoSHENTEK® *Mycobacteria* Real-Time PCR Detection

Introduction

The genus Mycobacterium contains around two hundred species including organisms that are classified from BSL-1 (biosafety level 1) to BSL-3. According to WHO, FDA, the European Pharmacopoeia and Chinese Pharmacopoeia regulations, cell banks, biological raw materials, harvests and products involved in the production of biologics should be examined for the absence of adventitious agents, including mycobacteria. WHO & FDA guidelines, the European Pharmacopoeia Chapter 2.6.16 and the Chinese Pharmacopoeia general chapter for quality control of animal cell substrate, recommended a validated NAT (Nucleic acid amplification test) method as an alternative to the culture method for the detection of mycobacteria contamination.

MycoSHENTEK® mycobacteria rapid detection qPCR kits (magnetic particle extraction + real-time PCR test) are used for qualitative detection of mycobacteria contamination in any biological starting materials and products, such as cell banks, cell culture, virus seeds, harvest, vaccines, cell-derived products, etc. It takes only six hours from sample preparation to qPCR assay results.

Product Number	Product Name	Quantity
1503601	MycoSHENTEK® Mycobacteria DNA Extraction Kit	50 Extractions
1503602	MycoSHENTEK® Mycobacteria DNA Detection Kit	50 Reactions

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Assay Validation

Parameters	Acceptance Criteria	
Inclusivity	The design of optimized multiplexed primers and reaction condition based on intensive bioinformatics analysis allows comprehensive detection of over 100 Mycobacteria species, avoiding detection of related bacterial species. 11 species of Mycobacteria microorganisms and 15 control plasmids containing Mycobacterial sequence were tested as validated PCR assays using MycoSHENTEK® kits.	
Sensitivity	Strain detection limit	The positive cut-off (≥95%), for LOD, for each of 14 Mycobacteria species was 10 CFU/mL.
	Genomic copy number sensitivity	The positive cut-off (≥95%), for sensitivity limit, was demonstrated for 10 types of positive-control plasmids containing Mycobacteria sequence, and determined as 20 copies/reaction.
Specificity	Sample matrix effect	Consistent performance in different sample matrices (e.g. serum, cell cryopreservation solution, BSA, etc.)
	Cross-reactivity	No cross-reactivity with more than 30 types of production cell substrate, engineered bacteria and fungi.
Robustness	Matrix robustness	All spiked samples in complex matrices (e.g. 10^7 cell/mL Vero/293T/CHO cell culture) gave positive results at the LOD level (10 cfu/mL).
	Freeze-thaw stability	Stable assay performance for at least 5 freeze-thaws cycles.
	Instrument suitability	Consistent assay performance on different qPCR instruments (e.g. SHENTEK-96S, ThermoFisher ABI 7500, Bio-Rad CFX96, Bioer FQD-96A, AnalytikJena qTOWER 3G & Roche LightCycler 480Ⅱ, etc.
	Experimental conditions	In all tests performed with slight variations of temperature cycling and reaction volume, the acceptance criteria were met for the samples and controls.
Repeatability	The intra-laboratory %CVs met the acceptance criteria (≤15%) for at a spike concentration of 100 CFU/mL.	

MycoSHENTEK® Mycobacteria Sensitivity Standards

SHENTEK® provides Mycobacteria sensitivity standards for the qPCR method validation. Each tube of sensitivity standard contains 10 CFU or 100 CFU of inactivated mycobacteria, which can be used safely and reliably in NAT methods. The colony forming unit (CFU) is determined by the culture method, and the genomic copy (GC) number by dPCR quantitation, are provided in the Certificate of Analysis for each lot. The standard cannot be used for culture method due to the inactivated mycobacteria. The standard solution or the matrix spike are intended for validating sensitivity, specificity, robustness, as well as the correlation check between the CT values and the log number of mycobacteria standard concentration.

Product Number	Product Name	Quantity
1503603	Mycobacterium phlei Sensitivity Standard (10 CFU)	3 tubes, 10 CFU/tube
1503604	Mycobacterium phlei Sensitivity Standard (100 CFU)	3 tubes, 100 CFU/tube
1503605	Mycobacterium neoaurum Sensitivity Standard (10 CFU)	3 tubes, 10 CFU/tube
1503606	Mycobacterium neoaurum Sensitivity Standard (100 CFU)	3 tubes, 100 CFU/tube

Quality Assurance

- The performance of MycoSHENTEK® Mycobacteria assay demonstrated rapid and reliable testing, with high sensitivity and specificity in compliance with pharmacopeial regulatory requirements, and is suitable for regulatory filing as an alternative detection method.
- Positive and internal controls are employed as references to prove the functionality of the reaction mix for amplification of the target and rule out inhibition.
- A premix of all components except the primers & probes is necessary to reduce pipetting steps, mitigate inconsistency risks and prevent sample contamination.
- The addition of dUTP/UNG to reaction mix eliminates carry-over contamination, the generation of false positives and inaccurate quantitation.
- Hot-Start DNA Polymerase and PCR buffer solution are optimized for increased resistance to various reaction inhibitors, providing increased specificity and higher yields of complementary DNA.
- The assays have been optimized to provide guaranteed performance, and reagents are stable under storage conditions for at least 2 years.

  Qualified production process is compliant with ISO13485 quality standard.