

The way
forward in
mycoplasma
detection.

Pharma analytics

MycoSEQ Mycoplasma Detection System



applied biosystems

Same-day results help ensure mycoplasma-free biotherapeutics

Rapid mycoplasma testing for lot-release and in-process testing

Mycoplasmas are the smallest known free-living organisms and are often referred to as a stealth invader or silent killer of high-value cell lines. Regulatory guidance requires that all products derived from mammalian cell culture be tested for the presence of mycoplasmas. Early and accurate detection of mycoplasma contamination aids in assuring compliance with regulatory guidance and helps protect against the spread of contamination into downstream processes and media.

The Applied Biosystems™ MycoSEQ™ Mycoplasma Detection System is an integrated, real-time PCR (qPCR) solution for rapid mycoplasma testing. Composed of functionally confirmed sample-to-answer protocols, commercially available kits for sample preparation, and qPCR instruments with dedicated analytical software to help enable regulatory compliance,

the MycoSEQ Mycoplasma Detection System and its workflow provide an easy-to-implement solution for a variety of mycoplasma testing applications.

- Uses a method accepted by regulatory agencies for mycoplasma detection in a Current Good Manufacturing Practice (CGMP) environment
- Requires **no live mycoplasmas** for testing or process validation
- Offers a total workflow solution, from sample prep to analysis, with security, audit, and e-signature (SAE)-compatible software
- Detects all recommended species listed in the European, US, and Japanese pharmacopeias
- Includes support throughout the validation process by our regulatory and field application specialists

The MycoSEQ Mycoplasma Detection System has been accepted worldwide to meet existing regulatory guidelines for the detection of mycoplasmas across multiple therapeutic modalities.

MycoSEQ Mycoplasma Detection System

Used throughout the bioproduction workflow, the MycoSEQ Mycoplasma Detection System provides an appealing alternative to time-consuming culture-based tests by delivering accurate and actionable results in about five hours. Backed by technical and regulatory professionals and a successful track record of regulatory acceptance, the MycoSEQ system can help simplify mycoplasma detection for raw material, cell bank, in-process, and lot-release testing.

Applied Biosystems™ AccuSEQ™
Real-Time PCR Software

Applied Biosystems™ QuantStudio™ 5
or 7500 Fast real-time PCR systems

Applied Biosystems™
AutoMate Express™
Nucleic Acid Extraction System



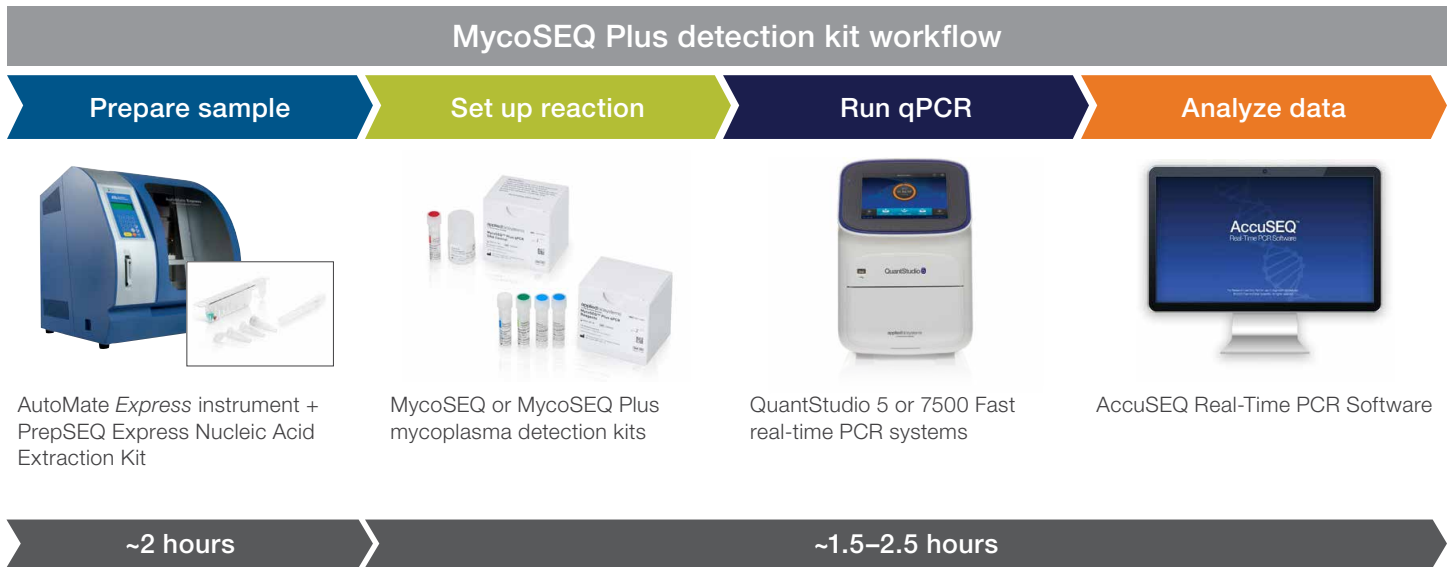
Applied Biosystems™ MycoSEQ™
mycoplasma detection assay kits

Applied Biosystems™ PrepSEQ™ Express
Nucleic Acid Extraction Kit
or PrepSEQ™ 1-2-3 Mycoplasma Nucleic Acid
Extraction Kit

Figure 1. The complete MycoSEQ Mycoplasma Detection System.

Sample-to-answer workflow delivers results in ~5 hours

The MycoSEQ Mycoplasma Detection System provides high performance and ease of use in an integrated, streamlined qPCR workflow. With complementary sample preparation protocols that support both manual and automated workflows, the MycoSEQ system can flex to meet your changing throughput needs. To enable optimal performance and system-wide integration, Thermo Fisher Scientific develops and tests all components of the system together.



Designed for purpose, confirmed by validation, and accepted by regulatory agencies

Real-time PCR-based assays provide a viable alternative to the culture-based method and provide results in hours while still meeting mycoplasma test performance guidance followed by regulatory bodies. Following validation, regulatory filing, and review, users worldwide and across multiple therapeutic modalities have received regulatory acceptance to use MycoSEQ mycoplasma detection assays* for lot-release testing.

The MycoSEQ Mycoplasma Detection System is designed to support the recommended qualification guidelines from the:

- International Conference on Harmonisation (ICH)
- United States Pharmacopeia (USP)
- European Pharmacopoeia (Ph. Eur.)
- Japanese Pharmacopoeia (JP)

Regulatory track record

As of 2021, the MycoSEQ system* has received regulatory acceptance in at least 42 commercially released drugs and was pending approval in 32 additional therapeutics.

More details on its regulatory track record can be found at thermofisher.com/mycoseq-validation.

* Legacy product Applied Biosystems™ MycoSEQ™ Mycoplasma Detection Assay.

The sample sets the stage for success

The success of a QC analytical test is critically dependent on the sample preparation used to purify and concentrate nucleic acids. We offer a range of sample prep solutions using reliable Applied Biosystems™ PrepSEQ™ chemistry. PrepSEQ nucleic acid extraction kits enable superior recovery efficiency for mycoplasma detection applications where consistent quantification and high sensitivity detection are required.

PrepSEQ nucleic acid extraction workflow

The PrepSEQ nucleic acid extraction workflow (Figure 1) employs our proprietary magnetic particle and binding solution that not only enables high binding but complete elution of the purified sample. The PrepSEQ solution offers flexible protocols for both manual and automated sample preparation, allowing you to process up to 13 samples at one time, with high recovery to help enable you to meet regulatory guidelines.



Figure 2. The PrepSEQ nucleic acid extraction workflow includes our proprietary magnetic particle and binding solution that not only enables high binding but complete elution of the purified sample.

PrepSEQ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit

Optimized for use with MycoSEQ mycoplasma detection kits, the PrepSEQ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit uses proprietary magnetic bead-based separation technology to extract *Mycoplasma* DNA from mammalian cell lines. The PrepSEQ 1-2-3 kit extracts *Mycoplasma* genomic DNA from research cell lines, stem cell lines, and cellular and tissue therapy samples with high efficiency and high recovery—delivering results in under 2 hours.



AutoMate Express Nucleic Acid Extraction System

An easy-to-use benchtop instrument enables hands-free automation of the nucleic acid extraction process for the MycoSEQ mycoplasma detection workflow. The instrument uses the established PrepSEQ nucleic acid extraction chemistry packaged into a cartridge format for quantitative recovery of DNA and RNA. The AutoMate Express system offers out-of-the-box automation for easy integration into existing workflows and lab configurations. There is minimal hands-on time with this closed system—just load and go with prefilled reagent cartridges and preprogrammed protocols. The AutoMate Express magnetic particle processor instrument offers low to medium throughput of up to 13 extractions in a single run.

Rapidly detect mycoplasmas with confidence

MycoSEQ mycoplasma detection kits are available in both probe-based and dye-based formulations to enable optimum performance, regardless of sample matrices.

MycoSEQ Plus Mycoplasma Detection Kit

The MycoSEQ Plus Mycoplasma Detection Kit is a probe-based quantitative PCR assay kit featuring gold-standard Applied Biosystems™ TaqMan™ chemistry. Developed specifically for the detection of mycoplasmas in cell therapy and complex bioproduction samples, the MycoSEQ Plus assay delivers performance that meets or exceeds regulatory requirements that support product lot-release testing, including the ability to meet regulatory guidance for assay sensitivity and specificity.

- Multiplexed assay enables simultaneous screening of over 200 species of mycoplasmas, with no demonstrated cross-reactivity to closely related bacterial species
- Proven sensitivity to meet or exceed a limit of detection (LOD) of less than or equal to 10 GC/mL for all *Mycoplasma* species listed in USP, Ph. Eur., and JP, as well as representative hemoplasmas, mesoplasmas, and ureaplasmas
- Limits risk to production facilities, as it does not require live mycoplasmas for testing or process validation
- Unique discriminatory positive control included with the assay can differentiate a control-based contamination event from a true positive sample call
- Presence of an internal positive control (IPC) confirms lack of PCR inhibition and PCR reaction consistency across all samples



MycoSEQ Mycoplasma Detection Kit



The tried-and-true MycoSEQ Mycoplasma Detection Kit sets the standard for rapid mycoplasma testing and has been validated in more than 42 therapeutics across a variety of modalities. The Applied Biosystems™ SYBR™ Green dye-based assay design utilizes a proprietary bioinformatics pipeline to detect >90 *Mycoplasma* species with high specificity and no cross-reactivity with closely related bacterial species. Its sensitivity has been demonstrated by internal and external validation methods to detect 10 CFU/mL, or the genomic equivalent of 10 GC/mL, as recommended by regulatory guidelines.

Compare MycoSEQ assays

	MycoSEQ Plus kit	MycoSEQ kit
Sample type	Bioproduction media, cells	Bioproduction media, cells
Therapeutic type	Cell therapies, gene therapies, viral vector-based therapies, biosimilars	Cell therapies, gene therapies, viral vector-based therapies, biosimilars
Detection chemistry	TaqMan chemistry (probe-based)	SYBR Green chemistry (dye-based)
Number of species detected	More than 200	More than 90
Discriminatory positive control	Yes	Yes
Internal positive control	Yes	No
Performance meets lot-release requirements	Yes	Yes

Simplicity, interactivity, and speed—choose your qPCR system

With more than 25 years of qPCR innovation, Thermo Fisher is a recognized leader in qPCR instrumentation. Applied Biosystems™ qPCR systems deliver true value with excellent performance, reliability, and world-class support. The following instruments have been internally validated with both the MycoSEQ kits and AccuSEQ software, helping ensure a smooth pathway to process qualification and validation.

QuantStudio 5 Real-Time PCR System

A high-performance benchtop instrument, the QuantStudio 5 Real-Time PCR System gives you greater control of your experimental data. Interact with the latest advancements in touchscreen usability, access your data more easily than ever before, and securely share your results.

- **Multiplex with ease**—six excitation filters and six emission filters offer 21 different color combinations, allowing a broad range of detection chemistries and maximum multiplexing
- **Have confidence in your records**—a built-in SAE package comes standard to assist you in 21 CFR Part 11 compliance
- **Protect your work**—individual user accounts and the ability to lock a protocol template give you peace of mind that only you can control your runs



7500 Fast Real-Time PCR System

An established qPCR workhorse, the 7500 Fast Real-Time PCR System is a powerful platform for labs requiring reliable performance with minimum investment. The 7500 Fast platform features an innovative optical system that enhances sensitivity and lets you access a broader range of fluorophores.

- Optional SAE package helps meet 21 CFR Part 11 compliance requirements
- An advanced multicomponent algorithm minimizes spectral crosstalk, making it excellent for multiplexing

Compare qPCR systems

	QuantStudio 5 system	7500 Fast system
Format	96-well; 0.1 mL	96-well; 0.1 mL
Sample ramp rate	3.66°C/sec	± 2.2°C/sec
Sensitivity	1 copy detection, 1.5 fold differences in target qty. (singleplex reaction)	
Dynamic range	10 logarithmic units	9 logarithmic units
Optical detection/multiplexing	6 decoupled filters, up to 6 targets/well	5 excitation filters, up to 5 targets/well
SAE	Included	Optional
Dimensions (H x W x D)	40 x 27 x 50 cm	49 x 34 x 45 cm
Display	Touchscreen	None
Connectivity	Wi-Fi enabled	None

Streamlined data interpretation for fast and accurate results

AccuSEQ Real-Time PCR Software offers efficient and streamlined data interpretation for analyzing contaminants and impurities in various testing scenarios. Serving as a pivotal element within an all-encompassing solution, AccuSEQ software equips you with actionable results crucial for biopharmaceutical manufacturing processes.

- Optimized and validated for all MycoSEQ assays
- Available for QuantStudio 5 and 7500 Fast qPCR systems
- Integrated and easy-to-use interface allows for consistency across multiple data applications
- A single software platform for all current and future MycoSEQ assays, as well as custom experiments
- SAE capabilities to help enable 21 CFR Part 11 compliance
- Complete traceability with assay-specific output, eliminating manual calculations
- Comprehensive data management and reporting features



Mycoplasma detection using AccuSEQ software

- **Predefined templates**—AccuSEQ software streamlines your experiment setup by providing predefined, factory-set templates for MycoSEQ, MycoSEQ Plus, and MycoSEQ Plus ΔRn tests, helping ensure you get up and running faster
- **Flexible call settings**—AccuSEQ software allows for great flexibility, with options to modify call settings to meet individual specifications, allowing customers to modify the thresholds for every call built as part of the decision tree in a presence/absence call (Figure 3); AccuSEQ software can also allow calls to be overridden, which can be done when you do not agree with the system calls and want to override it with your own assessment
- **Automated analysis**—Automated quantitation analysis and presence/absence calls based on a decision tree that utilizes parameters such as C_t , IPC Diff, and ΔRn (Figure 4); the analysis also features automated detection of PCR inhibition in sample wells

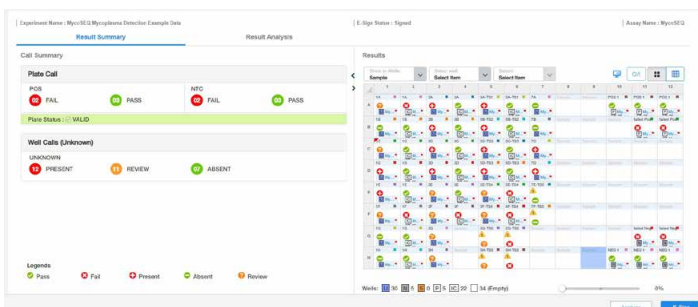


Figure 3. Automated presence or absence calls of mycoplasma contamination.

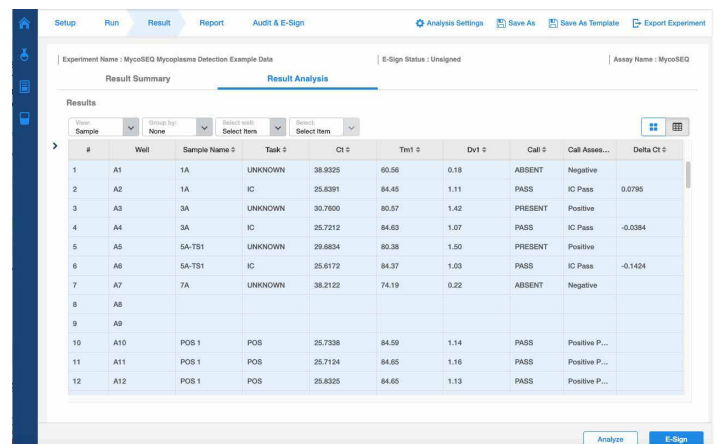


Figure 4. Results analysis table. Calls are made based on the three acceptance criteria: C, T, and the derivative value of test samples and inhibition controls.

Qualify with ease, use with success

To help ensure seamless integration into your lab, from initial process development and system implementation through validation and routine use, we will collaborate with your team to develop a comprehensive testing implementation plan.

This can include:

- Instrument installation with installation qualification (IQ)/operational qualification (OQ)
- Computer system validation (CSV)
- Method/protocol optimization
- Regulatory consulting and support
- Process validation guidance
- System and application training

Benefit from worldwide implementation and support

Our distribution and service network, composed of highly trained support and application personnel, reaches 150 countries on six continents.

Ordering information

Description	Cat. No.
Sample preparation	
PrepSEQ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit	4443789
PrepSEQ Express Nucleic Acid Extraction Kit	4466351
AutoMate <i>Express</i> Nucleic Acid Extraction System	4467754
MycoSEQ mycoplasma detection kits	
MycoSEQ Plus Mycoplasma Detection Kit (TaqMan chemistry)	A55124
MycoSEQ Plus Mycoplasma Detection Kit with PrepSEQ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit	A57925
MycoSEQ Plus Mycoplasma Detection Kit with PrepSEQ Express Nucleic Acid Extraction Kit	A57926
MycoSEQ Mycoplasma Detection Kit (SYBR Green chemistry)	4460623
MycoSEQ Mycoplasma Detection Kit with PrepSEQ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit	4460626
Real-time PCR instruments	
QuantStudio 5 Real-Time PCR System, 96-well, 0.1 mL, w/laptop (Pharmaceutical Analytics)	A31671
7500 Fast Real-Time PCR System, w/tower (QST)	4365463
Data analysis	
AccuSEQ Real-Time PCR Detection Software v3.2 (for QuantStudio 5 instrument)	A58643
AccuSEQ Real-Time PCR Detection Software v2.2 (for 7500 Fast instrument)	A58642

Learn more at thermofisher.com/mycoseq

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