ThermoFisher SCIENTIFIC

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™ 7 Pro, 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.

MycoSEQ Plus detection kit workflow

Prepare sample	Set up reaction	Run qPCR	Analyze data
			
AutoMate *Express* instrument + PrepSEQ Express Nucleic Acid Extraction Kit	MycoSEQ or MycoSEQ Plus mycoplasma detection kits	QuantStudio 7 Pro, QuantStudio 5, or 7500 real-time PCR systems	AccuSEQ Real-Time PCR Software
~2 hours	~1.5–2.5 hours		

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 help 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 you meet regulatory guidelines.

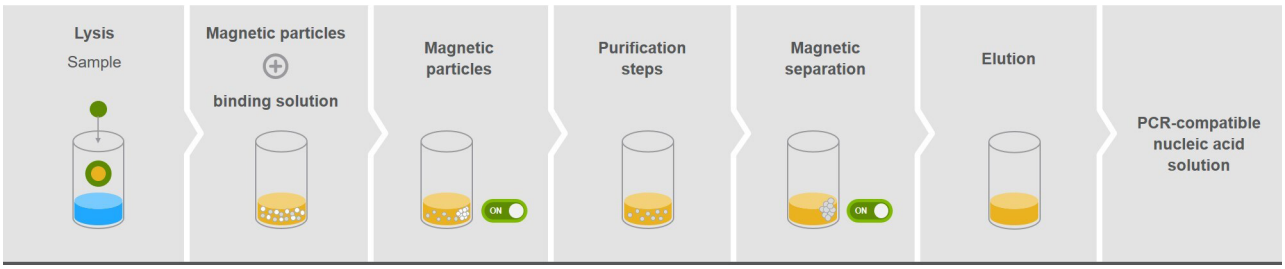

Figure 2. The PrepSEQ nucleic acid extraction workflow includes our proprietary magnetic particle and binding solution that not only supports high binding but complete elution of the purified sample.

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 7 Pro Real-Time PCR System

The QuantStudio 7 Pro system offers exceptional flexibility to support various applications and throughput needs. Designed with smart features to reduce contamination, it allows hands-free setup and remote scheduling and monitoring from your mobile device.

- **Adapt fast and accelerate processes**—interchangeable blocks enable increased throughput and offer enhanced convenience and adaptability, accommodating 96-well, 384-well, and TaqMan array cards
- **Increase data integrity and traceability**—advanced SAE features, including SAE-specific archive and restore capabilities, assist with 21 CFR Part 11 compliance, limit unauthorized access, and help monitor data-impacting events
- **Reduce manual tasks**—automation compatibility supports 24/7 operation and the ability to add automatic loading systems
- **Ease user access and more**—Lightweight Directory Access Protocol (LDAP) support streamlines user access with single sign-on capability and a single audit trail for both the instrument and the software makes it easy to track users and system events
- **Stay secure**—customizable user roles and permissions enhance security while promoting workflow efficiency and enabling greater accountability and traceability
- **Stay informed**—system and email notifications improve operational productivity and help optimize resource utilization by alerting you to system status changes

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

Sales of 7500 Fast systems will be discontinued as of October 31, 2025 and full service and support availability will be discontinued by October 31, 2030. If you're currently using a 7500 Fast system, you can leap forward to a smarter, faster, and more efficient testing experience by upgrading your qPCR system with our trade-in program.

To get started and request a quote, visit **thermofisher.com/qpcrtradein**

Compare qPCR systems

	QuantStudio 7 system	QuantStudio 5 system	7500 Fast system
Format	96-well; 0.1 mL, 0.2 mL, and 384-well	96-well; 0.1 mL	96-well; 0.1 mL
Sample ramp rate	3.66°C/sec.	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	10 logarithmic units	9 logarithmic units
Optical detection/multiplexing	6 decoupled filters, up to 6 targets/well	6 decoupled filters, up to 6 targets/well	5 excitation filters, up to 5 targets/well
SAE package	Included with enhanced functionality (not compatible with QuantStudio 5)	Included	Optional
Dimensions (H x W x D)	54.7 x 33.8 x 52.5 cm	40 x 27 x 50 cm	49 x 34 x 45 cm
Display	12-in. capacitive touchscreen with real-time application viewing	Touchscreen	None
Connectivity	WiFi enabled, Thermo Fisher Connect Platform (cloud) enabled	Wi-Fi enabled	None
Automation compatibility	Yes	No	No

Take control of your data with one powerful software solution

AccuSEQ Real-Time PCR Software offers rapid and straightforward data interpretation for contaminant and impurity testing. As a crucial part of a comprehensive solution, it delivers actionable insights essential for biopharmaceutical manufacturing. AccuSEQ software integrates with a validated workflow, encompassing sample preparation, real-time PCR, data analysis, and report generation for precise contaminant and impurity assessment.

AccuSEQ software offers an efficient, cost-effective approach to real-time PCR data analysis by helping you establish processes suited to your specific requirements, take advantage of advanced QuantStudio qPCR instrumentation, and strengthen data protection and regulatory compliance.

AccuSEQ software features

- Optimized and validated for MycoSEQ assays
- An integrated, easy-to-use interface to enable consistency across multiple data applications, reducing the time needed to train your team
- SAE capabilities support 21 CFR Part 11 compliance and include username and password restrictions, audit trails tracked through the SAE module, and a range of e-signature modes and actions
- Flexible throughput of 10–500 samples per week
- Traceability with assay-specific output, helping eliminate 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

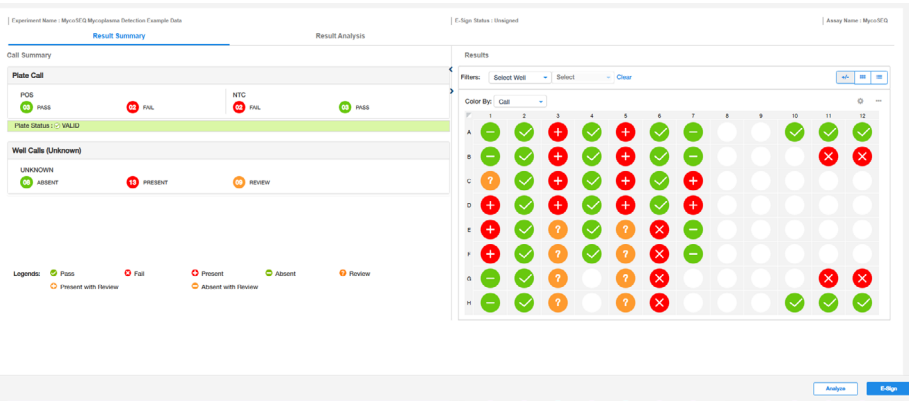

Figure 3. Automated presence or absence calls of mycoplasma contamination.

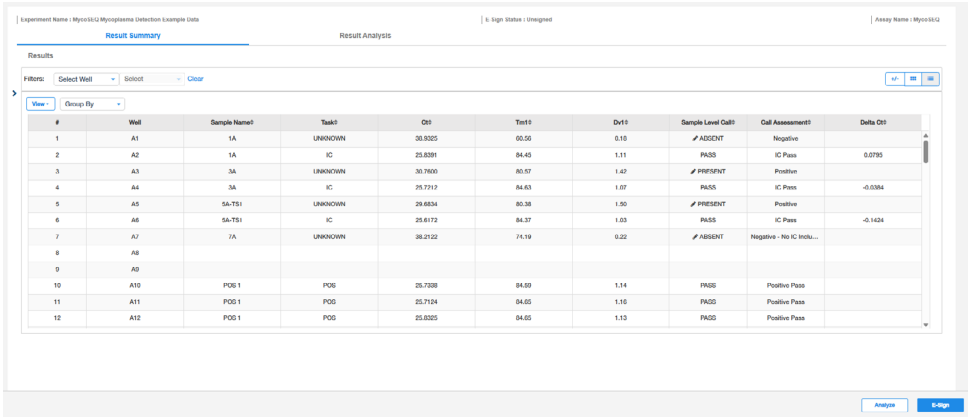

Figure 4. Results analysis table.
Calls are made based on the three acceptance criteria: C, T, and the derivative value of test samples and inhibition controls.

Take control of your timeline
By your side from evaluation to validation

Releasing your product safely, rapidly, and cost-effectively is your top priority—and we're here to help. Thermo Fisher Scientific services can help maximize the utility and effectiveness of your MycoSEQ system. Whether it's setting up instruments and equipment, installing and fine-tuning software, optimizing your lab or site, or training your staff, we can get you up and running quickly and efficiently.

Design and implementation
Save time and money by getting your lab designed and set up efficiently the first time.

- Laboratory workflow consultation and setup assistance based on good molecular biology lab practices
- Introduction to the basics of DNA-based vs. traditional culture methods
- Multiple visits by a specialist over a six-month period
- Guidance in establishing data acceptance criteria and interpretation
- Detailed analysis of the data collection to identification process
- Workflow design to maximize efficiency for variable sample throughput
- Software training and routine system maintenance

Installation and operation qualification (IQ/OQ)
Get your lab up and running in record time so you can validate your system and reach routine use.

- Determine training needs, system installation, and validation timelines and plans
- System hardware and software installation and operation
- Software security and audit trail verification
- IQ protocol and service: as fast as 2 days
- OQ protocol and service: as fast as 3 days
- Performance qualification (PQ) recommended guidelines

Computer system validation (CSV)
If it isn't documented, then it can't be proven—but CSV provides the evidence you need.

- Documentation that a computer system meets a set of defined requirements
- Documentation of user requirements for the software
- Verification that electronic record-keeping systems are performing to specifications
- Audit-style template documentation for development and flexibility
- Validation of each system's ability to identify altered or invalid records

Comprehensive training
Train your team quickly with our one- or two-day training options.

- Online learning delivered by specialists, right at your desktop
- On-site instruction from factory trained professionals
- Courses at one of our global training and demo centers

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 *Express* 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
QuantStudio 5 Real-Time PCR System, 96-well, 0.1 mL, w/desktop (Pharmaceutical Analytics)	A31672
QuantStudio 7 Pro Real-Time PCR System, 96-well, 0.1 mL, w/laptop (Pharmaceutical Analytics)	A40006420
QuantStudio 7 Pro Real-Time PCR System, 96-well, 0.1 mL, w/tower (Pharmaceutical Analytics)	A40006426
Data analysis	
AccuSEQ Real-Time PCR Detection Software v4.0	A40005162
AccuSEQ Real-Time PCR Detection Software v4.0 (upgrade license)	A40005161
AccuSEQ Real-Time PCR Detection Software v4.0 (seat license)	A40005163

Learn more at **thermofisher.com/mycoseq**