

Pharma analytics

MycoSEQ Plus Mycoplasma Detection Kit

Rapid mycoplasma detection for bioproduction sample testing

Introduction

Mycoplasmas are the smallest known free-living organisms and are often referred to as the 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. Figure 1 shows the different steps in biopharmaceutical manufacturing where testing for mycoplasmas is typically performed.



MycoSEQ Plus Mycoplasma Detection Kit

The Applied Biosystems™ MycoSEQ™ Plus Mycoplasma Detection Kit is a probe-based quantitative PCR (qPCR) solution for rapid mycoplasma detection using gold-standard Applied Biosystems™ TaqMan™ chemistry. Developed specifically for detection of mycoplasmas in cell therapy and complex bioproduction samples, MycoSEQ Plus assays deliver performance that meets or exceeds regulatory requirements while helping to preserve valuable cells needed for treatment.

The kit enables simultaneous screening of more than 200 *Mycoplasma* species, with no cross-reactivity to closely related bacterial species or impurities from production methods, such as genomic DNA from a mammalian cell culture-based production project (Tables 1 and 2). Furthermore, the unique discriminatory positive control allows differentiation between a control-based contamination event from a true positive sample.

- **Sensitive**—meets or exceeds the recommended regulatory guidance of 10 CFU/mL or 10 genomic copies (GC)/mL for a wide variety of mycoplasma species, including those commonly known to contaminate bioprocessing cultures
- **Specific**—no observed cross-reactivity to closely related non-mycoplasma bacterial species (e.g., *Clostridium*, *Lactobacillus*, *Streptococcus* spp.)
- **Accurate**—discriminatory positive control allows discrimination between true mycoplasmas and accidental contamination of test sample with positive control
- **Fast**—qPCR-based workflow delivers actionable results in less than 5 hours, enabling quick confirmation of mycoplasma-free production harvest to help expedite the release of your product
- **Comprehensive**—multiplex assay enables simultaneous screening of >200 mycoplasma species, including all key species listed in the US, European, and Japanese pharmacopeias (USP, EP, and JP)

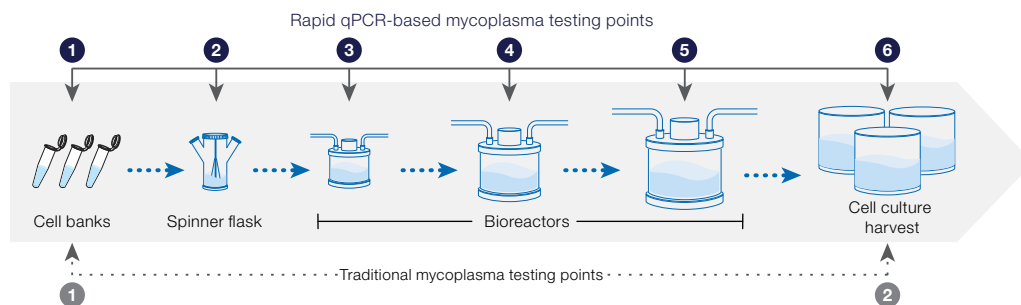


Figure 1. Sampling points for mycoplasmas. Rapid qPCR-based testing for mycoplasma contamination can be conducted throughout the cell culture manufacturing process, from inoculation through harvest.

Table 1. Specifications of the MycoSEQ Plus Mycoplasma Detection Kit.

Sample types	Bioproduction media, cells
Therapeutic types	Cell therapies, mAbs, vaccines, biosimilars, and gene therapies
Detection chemistry	TaqMan probe-based qPCR
Sensitivity; limit of detection (LOD)	≤10 GC/mL
Number of species	>200 species including confirmed functional testing of species in the USP, EP, and JP, along with representative <i>Hemoplasma</i> , <i>Mesoplasma</i> , and <i>Ureaplasma</i> species
Number of reactions per kit	100
Hours per workflow	≤5 hours
Controls	Discriminatory positive control (DPC); internal positive control (IPC)
PCR inhibition	No inhibition observed in presence of potential PCR inhibitors: anti-clumping agent, EGTA, heparin
Cell line DNA	No cross-reactivity observed with production workflow components (e.g., human, CHO, NS0, Vero, lentivirus)
Sample matrices	CHO and T cell media; cells and media; bioreactor CHO cell samples

Table 2. Partial panel of species detected by the MycoSEQ Plus Detection Assay. The MycoSEQ Plus kit has been tested to detect ≤10 GC/mL of all species referenced in the European, US, and Japanese pharmacopeias along with representative *Hemoplasma*, *Mesoplasma*, and *Ureaplasma* species.

Species detected by the MycoSEQ Plus kit	EP	USP	JP
<i>Mycoplasma hyorhinis</i>	•	•	•
<i>Acholeplasma laidlawii</i>	•	•	•
<i>Mycoplasma orale</i>	•	•	•
<i>Mycoplasma pneumoniae</i>	•	•	•
<i>Mycoplasma fermentans</i>	•	•	•
<i>Mycoplasma synoviae</i>	•	•	
<i>Mycoplasma gallisepticum</i>	•	•	
<i>Spiroplasma citri</i>			•
<i>Mycoplasma arginini</i>	•		•
<i>Mycoplasma salivarium</i>			•
<i>Mycoplasma haemohominis</i>			
<i>Mycoplasma wenyonii</i>			
<i>Mesoplasma tabanidae</i>			
<i>Mycoplasma hominis</i>			
<i>Mycoplasma mycoides</i>			
<i>Mycoplasma pirum</i>			
<i>Ureaplasma urealyticum</i>			

The MycoSEQ Plus Mycoplasma Detection Kit includes:

- MycoSEQ Plus 10X assay mix
- TaqMan chemistry-based master mix
- Discriminatory positive control
- Negative control water
- DNA dilution buffer
- Complete protocol for test setup and data analysis

Gold-standard performance with TaqMan technology

The MycoSEQ Plus assay uses highly sensitive TaqMan probes that incorporate a 5′ fluorescent reporter dye and a 3′ nonfluorescent quencher (NFQ). The fluorogenic probe is specific to the target gene while the NFQ offers the advantage of lower background signal. Due to the specificity of TaqMan technology, the assay is able to distinguish between different targets within the same reaction, allowing multiple types of controls to be included in the test design (Figure 2).

Integrated controls enabling increased accuracy

The MycoSEQ Plus Mycoplasma Detection Kit includes a proprietary discriminatory positive control (DPC) designed to behave like mycoplasma DNA in both the sample preparation and detection steps of the assay. The DNA sequence of the DPC has been modified to distinguish between mycoplasma and control DNA, as shown by the distinctive labels associated with each component (Figure 2). This novel design enables risk-free DNA spike control testing, minimizing the possibility of mistaking a false-positive call due to an accidental cross-contamination with the DPC for a true positive mycoplasma test result.

Additionally, the MycoSEQ Plus kit features an internal positive control (IPC) that serves as a PCR inhibition control and provides confirmation of consistent performance across all reactions (Figure 2).

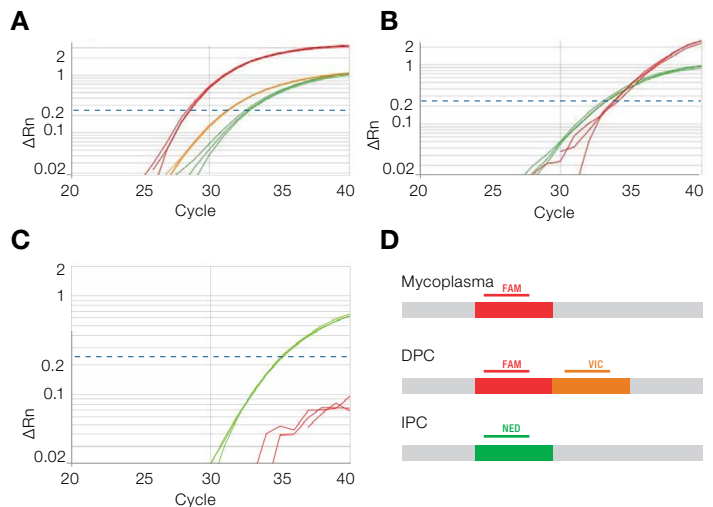


Figure 2. Multi-level controls confirm performance. qPCR amplification plots depict representative results from: (A) positive control, using 2,000 copies of the discriminatory positive control (DPC), (B) simulated mycoplasma contamination (10 copies), using *M. arginini* gDNA extracted from spent Gibco™ CTS™ medium with T cells, and (C) negative control, using spent Gibco CTS medium with T cells. (D) Reporter signals associated with mycoplasma and control constructs (DPC and IPC). The qPCR threshold is set at 0.25 (dashed blue line).

Integrated workflow delivers actionable results in less than 5 hours

The MycoSEQ Plus kit is part of an integrated qPCR-based detection system that greatly simplifies implementing mycoplasma testing into your manufacturing process. The system offers both manual and automated sample prep solutions, industry-leading Applied Biosystems™ real-time PCR instruments, and SAE-compatible analysis software offering predefined templates and features to help with data interpretation, such as automated reporting of presence and absence.

The easy, streamlined workflow typically delivers actionable results in less than 5 hours (Figure 3), enabling early detection of mycoplasma contamination, while the flexible throughput supports fluctuating testing needs and enables reduced cost per reaction through batch-testing multiple samples.

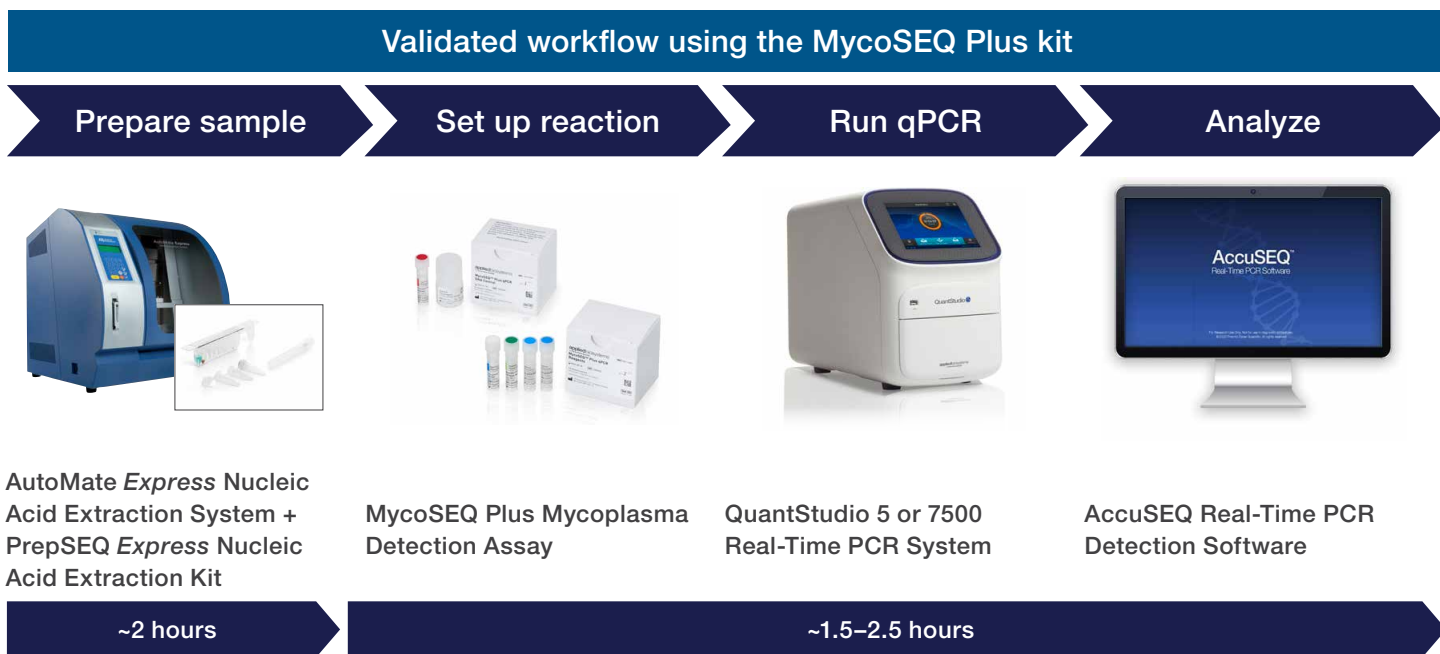


Figure 3. Integrated workflow solution to support in-process testing and final lot release. The MycoSEQ Plus kit is part of an integrated workflow for contaminant testing during biopharmaceutical manufacturing.

PrepSEQ mycoplasma sample preparation kits

Available with the MycoSEQ Plus kit, Applied Biosystems™ PrepSEQ™ mycoplasma sample preparation kits are optimized for highly efficient DNA recovery for mycoplasma detection. PrepSEQ kits use proprietary magnetic bead-based separation technology to extract mycoplasma DNA from mammalian cell culture samples with high efficiency, and offer the flexibility to process from 100 µL to 10 mL of cell culture containing as many as 10⁸ cells. The Applied Biosystems™ PrepSEQ™ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit is used for manual processing methods. An automated approach is also available using the Applied Biosystems™ PrepSEQ™ Express Nucleic Acid Extraction Kit. The Applied Biosystems™ AutoMate Express™ Nucleic Acid Extraction System is based on prefilled sealed cartridges, providing walk-away nucleic acid extraction within a fully enclosed system.

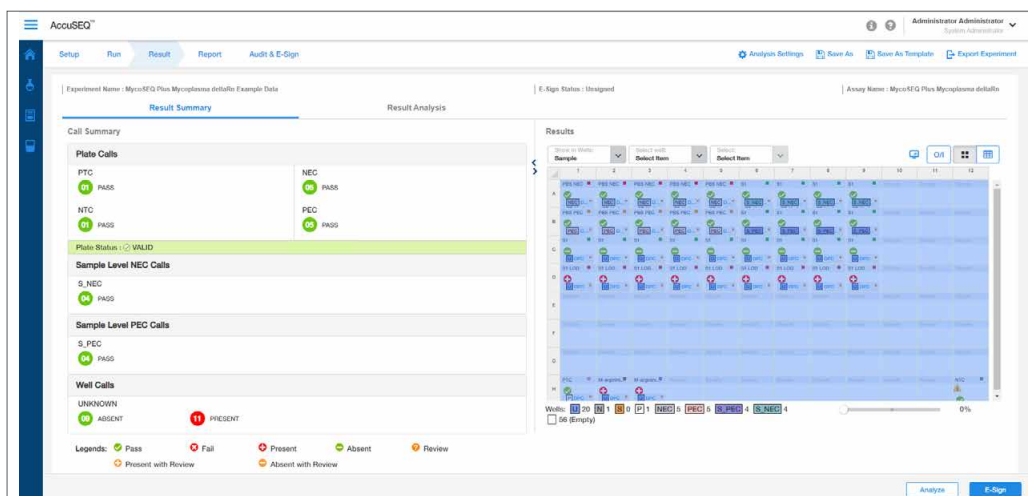
The customizable protocol design can accommodate a wide variety of sample types. The following samples have been internally tested with PrepSEQ kits:

- High-titer CHO cultures from bioreactors
- High-titer NS0 cultures from bioreactors
- Cell culture harvest for vaccine manufacturing
- Transgenic milk
- Bioassay cell lines
- Stem cell cultures
- Lymphocyte proliferation cultures for autologous transplantation
- Cell and tissue therapy cultures
- Sera
- Cell culture media

AccuSEQ Real-Time PCR Detection Software for automated mycoplasma data analysis

Automated presence or absence results from the MycoSEQ Plus Mycoplasma Detection Kit can be generated using Applied Biosystems™ AccuSEQ™ Real-Time PCR Detection Software. Advanced algorithms for this automated calling were developed using data interpretation guidelines for the MycoSEQ Plus Mycoplasma Detection Assay. Automated analysis tools enable one-click processing of MycoSEQ assay data, helping to deliver presence or absence calls within seconds of data

collection being completed. For in-depth review of the data, AccuSEQ software offers easy-to-use manual review tools, including a complete table of C_t values as well as amplification, multicomponent, and raw data plots. Security, audit, and e-signature (SAE) capabilities included with AccuSEQ software help enable compliance with 21 CFR Part 11. AccuSEQ software is available for both Applied Biosystems™ QuantStudio™ 5 and 7500 Fast Real-Time PCR Systems.



Ordering information

Product	Quantity	Cat. No.
Mycoplasma detection kits		
MycoSEQ Plus Mycoplasma Detection Kit	100 reactions	A55124
MycoSEQ Plus Mycoplasma Detection Kit with PrepSEQ 1-2-3 Nucleic Acid Extraction Kit	100 reactions	A57925
MycoSEQ Plus Mycoplasma Detection Kit with PrepSEQ Express Nucleic Acid Extraction Kit	100 reactions	A57926
Sample preparation		
PrepSEQ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit	100 extractions	4443789
PrepSEQ Express Nucleic Acid Extraction Kit	52 extractions	4466351
AutoMate Express Nucleic Acid Extraction System	1 instrument	4467754
Real-time PCR systems		
Pharmaceutical Analytics QuantStudio 5 Real-Time PCR System, 96-well, 0.1 mL, laptop	1 instrument	A31671
7500 Fast Real-Time PCR System, tower, QST	1 instrument	4365463
Analysis software		
AccuSEQ Real-Time PCR Detection Software v2.2 (QuantStudio 5 system)	1 license	A58642
AccuSEQ Real-Time PCR Detection Software v3.2 (7500 Fast system)	1 license	A58643

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