

USER GUIDE

applied
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by *life* technologies™

MycoSEQ™ Mycoplasma Detection Kits

MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit

MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit

Publication Part Number 4465874 Rev. A

Revision Date August 2011

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technologies™

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About This Guide

IMPORTANT! Before using this product, read and understand the information the “Safety” appendix in this document.

Purpose

This guide provides:


- Background information about the detection of *Mycoplasma* species
- A list of materials and equipment that can be used with the MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit and the MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit
- Guidelines for sample preparation
- Instructions for preparing reaction plates and performing PCR using the MycoSEQ™ *Mycoplasma* Detection Kits on Applied Biosystems Real-Time PCR Systems
- General troubleshooting guidelines


User attention words


Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

 **CAUTION!** Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

 **WARNING!** Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

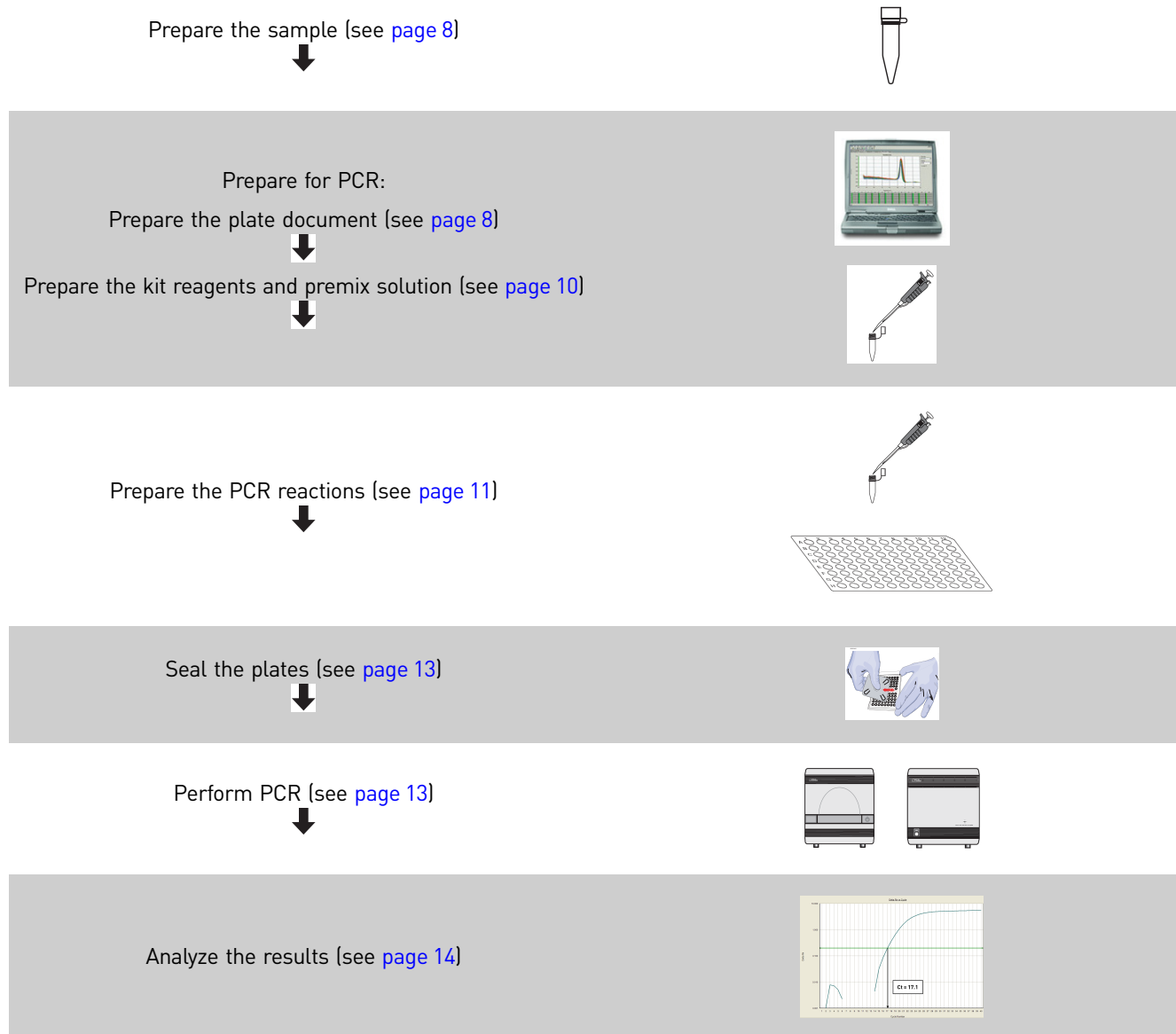
 **DANGER!** Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit

This protocol applies to both the MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit and the MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit.

IMPORTANT! For information on how to avoid PCR contamination, see [Appendix C on page 27](#).

Kit workflow



Prepare the sample

Refer to the *PrepSEQ® Sample Preparation Kits User Guide* (PN 4465957) for details on sample preparation.

Prepare for PCR

Prepare the plate document

Set up the plate document in the SDS software. For more details, refer to the *7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide* or the *7900HT Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide*:

1. In the Assay drop-down list, select **Absolute Quantification**.
2. Select SYBR® detector with:
 - Quencher Dye set to **(none)** or **(Non Fluorescent)**
 - Passive Reference set to **ROX**
3. Set thermal-cycling conditions as indicated in the table below.

Note: For instruments using the AccuSEQ® Real-Time PCR Software *Mycoplasma* Module, the cycling conditions are pre-programmed in the software.

Step	AmpliTaq Gold® enzyme activation	PCR		Dissociation†‡§			
		Denature	Anneal/extend	Melt			
	HOLD	Cycle (40 cycles)					
Temp	95 °C	95 °C	60 °C	95 °C	60 °C	95 °C	60 °C
Time	10 min	15 sec	1 min	15 sec	1 min	15 sec	15 sec

† 7500 and 7500 Fast Systems: from the Instrument tab, click **Add Dissociation Stage** (see [Figure 1 on page 9](#)).

‡ (Optional) 7900HT Fast Systems: from the Instrument tab, click **Add Dissociation Stage**, then click **Add Step** (to set the four temperatures required during the Dissociation Stage; see [Figure 2 on page 9](#)).

§ For other instruments, refer to their corresponding user guides for dissociation-curve setup information.

4. Set Sample Volume to **30 µL**.
5. Select the appropriate Run Mode for use with SYBR® Green I dye:
 - **For the 7500 Fast system** – select **Standard 7500** Run Mode.
 - **For the 7900HT Fast system** – select **Standard** Run Mode.

Figure 1 The instrument tab for 7500 Fast Time Real-Time PCR platform with SDS v1.4 or v1.5 21 CFR Part 11 software. The run mode is set to Standard 7500.

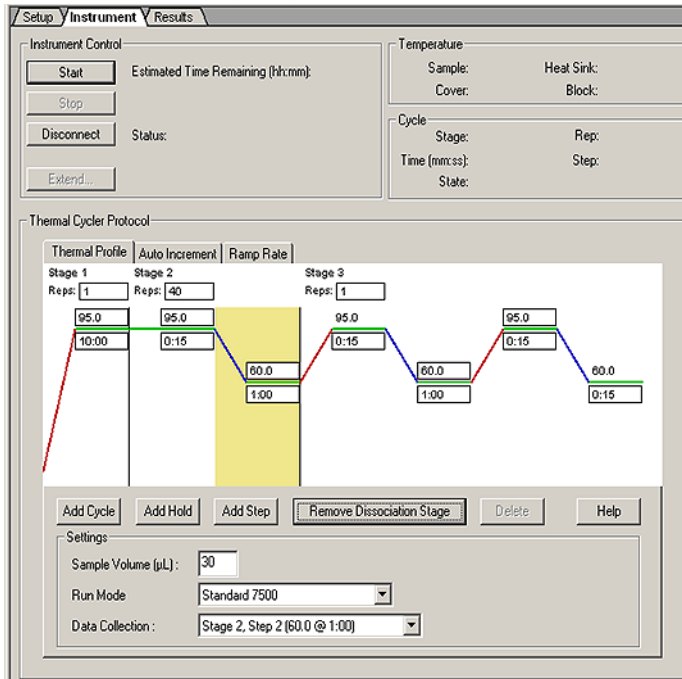
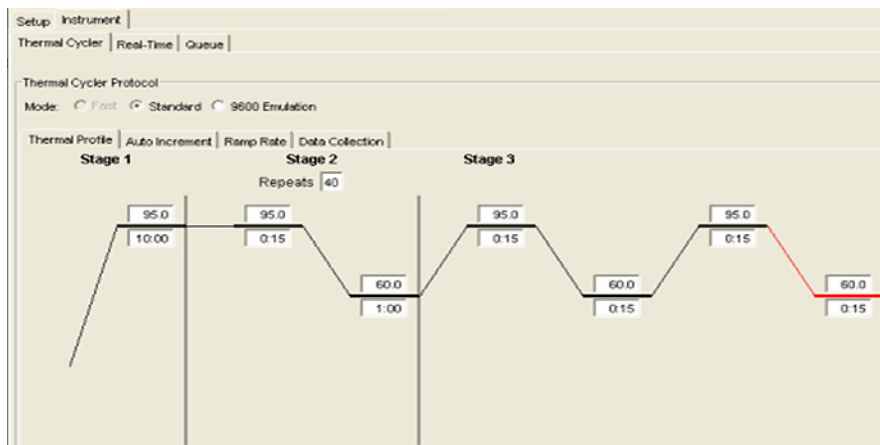


Figure 2 The instrument tab for 7900HT Fast platform with SDS 2.3 21 CFR Part 11 software. The run mode is set to Standard.



Prepare the kit reagents and premix solution

1. Thaw all kit reagents completely. Applied Biosystems recommends thawing the positive control at 37 °C for 5 minutes to ensure consistent results.
2. Vortex, then spin down the reagents.
3. Prepare the Premix Solution according to the following table.

Component for premix solution	Volume for one 30-µL reaction (µL)	Volume for four 30-µL reactions (µL) [†]
Power SYBR® Green PCR Master Mix (2X) or Myco Scan Power SYBR® Green PCR Master Mix (2X)	15.0	66.0
<i>Mycoplasma</i> Real-Time PCR Primer Mix (10X) or Myco Scan <i>Mycoplasma</i> Real-Time PCR Primer Mix (10X)	3.0	13.2
Total premix solution volume	18.0	79.2

[†] Includes 10% excess to compensate for pipetting errors.

4. Mix the Premix Solution by gently pipetting up and down, then cap the tube.

Prepare the PCR reactions

Guidelines for using the MycoSEQ™ Discriminatory Positive/Extraction controls

1. Pipet the reagent volumes into labeled microcentrifuge tubes or the wells of a reaction plate using the following table as a guide:

To prepare...	In each tube or well...
Negative-control reaction	<ul style="list-style-type: none"> • Add 18 µL of Premix Solution • Add 12 µL of Negative Control (water)
Your unknown sample reaction	<ul style="list-style-type: none"> • Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of Negative Control (water)
Inhibition-control reaction	<ul style="list-style-type: none"> • Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of the Discriminatory Positive Control (DPC)
Positive-control reaction	<ul style="list-style-type: none"> • Add 18 µL of Premix Solution • Add 2 µL of the DPC • Add 10 µL of Negative Control (water)

Note: The MycoSEQ™ *Mycoplasma* Discriminatory Positive/Extraction Control (Part Number 4445000) can be used as a spike control that is added to the test sample or lysate prior to sample preparation

2. Dispense 18 µL of Premix Solution into each well to be used, gently pipetting at the bottom of the well. For the:
 - **7500 Fast system** – Dispense into a Fast optical 96-well plate (PN 4346906).
 - **7500 and 7900HT Fast (standard block) systems** – Dispense into a standard optical 96-well plate (PN 4306737).
 - **7900HT Fast system (Fast block)** – Dispense into a Fast optical 96-well plate (PN 4346906).
3. For each row of wells that you use, place in sequence from left to right the negative control, unknown sample, inhibition control, then positive control. See [Figure 3, “Example plate layout” on page 12](#) and [“Plate layout suggestions” on page 28](#) for more information.

Pipetting guidelines:

- Use at least one negative and one positive control per run.
- Mix each sample very gently by pipetting up and down.
- Use a new tip for each well, even when aliquoting the same solution.

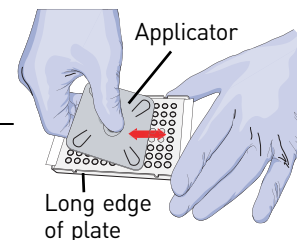
Figure 3 Example plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NEG 1 N Mycopl				Sample 1 U Mycopl				Sample 1 IC Mycopl			POS 1 P Mycopl
B					Sample 2 U Mycopl				Sample 2 IC Mycopl			
C					Sample 3 U Mycopl				Sample 3 IC Mycopl			
D					Sample 4 U Mycopl				Sample 4 IC Mycopl			
E					Sample 5 U Mycopl				Sample 5 IC Mycopl			
F					Sample 6 U Mycopl				Sample 6 IC Mycopl			
G					Sample 7 U Mycopl				Sample 7 IC Mycopl			
H					Sample 8 U Mycopl				Sample 8 IC Mycopl			

Wells: U Unknown 8 N Negative Control 1 P Positive Control 1 IC Inhibition Control 8 78 Empty

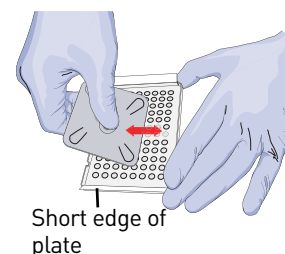
Seal the plates

1. Place an optical adhesive cover on the plate, then rub the flat edge of the applicator back and forth along the *long* edge of the plate.

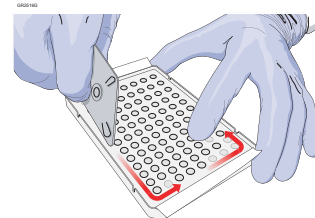


IMPORTANT! Apply significant downward pressure on the applicator to completely seal the wells. Pressure is required to activate the adhesive on the optical cover.

2. Rub the flat edge of the applicator back and forth along the *short* edge (width) of the plate.



3. Rub the edge of the applicator horizontally and vertically between all wells.
4. Rub the edge of the applicator around all outside edges of the plate using small back and forth motions to completely seal around the outside wells.



5. Vortex the plate on the low setting for 5 seconds. If you see liquid on the well sidewalls, spin down the plate at $2000 \times g$ for 20 seconds using a centrifuge with a plate adapter.

IMPORTANT! Make sure that the reagents are in the bottom of the wells.

Perform PCR

On an Applied Biosystems Real-Time PCR System:

1. Open the plate document that corresponds to the reaction plate ("[Prepare the plate document](#)" on page 8).
2. Load the reaction plate into the real-time PCR system.
3. Start the run.

Analyze the results

The acceptance criteria provided in this section are based on our current knowledge of assay performance in detection of *Mycoplasma* recovered from a wide variety of test sample matrices. We recommend that you qualify and validate the assay internally using samples that are specific to your process and manufacturing environment (raw materials, bioreactor or cell line samples) in order to verify that these criteria are appropriate. For specific sample types, it may be necessary to make slight adjustments to the acceptance criteria based on specific results. Life Technologies can provide you with one-on-one support during this process.

Set the baseline and threshold values

For all reactions, use the default Analysis Settings:

1. Select Manual C_T , then set Threshold to **0.2**.
2. Select **Manual Baseline**, then enter the following settings:
 - Start (cycle): **3**
 - End (cycle): **15**

Guidance for test samples

The table shows criteria for positive and negative calls. A positive call indicates that at least one genome copy of *Mycoplasma* DNA was present in the test reaction and the sample is positive for the presence of *Mycoplasma*. The automated threshold setting for derivative value (DV) of 0.8 for AccuSEQ® is equivalent to the 0.05 setting for SDS v1.4 software.

Table 1 Criteria for test samples: AccuSEQ® Real-Time PCR Detection Software

Result	C_T	T_m	DV
Positive	< 36.23	75°C – 85°C	≥ 0.8
Negative	≥ 36	75°C – 85°C	< 0.8

Table 2 Criteria for test samples: SDS software v1.4

Result	C_T	T_m	DV
Positive	< 36	75°C – 81°C	≥ 0.05
Negative	≥ 36	75°C – 81°C	< 0.05

Guidance for controls

Table 3 Criteria for controls: AccuSEQ® Real-Time PCR Detection Software


Control	C_T	T_m	DV
PCR positive control	<36.23	≈ 84°C	> 0.8
Extraction spike control	<36.23	≈ 84°C	> 0.8
No template control	≥36.23	< 75°C	< 0.8
Blank extraction control	≥36.23	< 75°C	< 0.8
Inhibition control	$\Delta C_T < 2$	~ 84°C	> 0.8



Table 4 Criteria for controls: SDS software v1.4

Control	C _T	T _m	DV
PCR positive control	< 36	≈ 84°C	> 0.05
Extraction spike control	< 36	≈ 84°C	> 0.05
No template control	≥ 36	< 75°C	< 0.1
Blank extraction control	≥ 36	< 75°C	< 0.1
Inhibition control	ΔC _T < 2	≈ 84°C	> 0.05

- Both the PCR positive control and the extraction spike control may present extra peaks with T_m < 75°C. These peaks represent primer dimer formation, and they do not interfere with the final results.
- The difference in C_T between the DPC and the inhibition control reaction should be less than 2. If the unknown sample is negative and the inhibition control shows a ΔC_T > 2 when compared to the positive control, then the PCR is likely inhibited. The sample should be re-purified and the assay repeated.

Guidance for inconclusive results with AccuSEQ® software

If a MycoSEQ™ assay does not meet all of the criteria for a positive or negative automatic call, the well displays  (inconclusive). For information about these results:

- Click  Quality Summary (Quality Summary) in the Results navigation pane of the AccuSEQ® software screen.
- Click  (Help) in the toolbar at the top of the AccuSEQ® software screen.
- See “[Troubleshooting](#)” on page 19.
- Refer to the *AccuSEQ® Real-Time PCR Detection Software Mycoplasma SEQ Experiments Getting Started Guide*.

Example results with SDS v1.4 software

Note: If you are using AccuSEQ® Real-Time PCR Software, refer to the *AccuSEQ® Real-Time PCR Detection Software Mycoplasma SEQ Experiments Getting Started Guide* for more data analysis information and example results.

The graphs below show examples of results from analysis with SDS v1.4 or v1.5 21 CFR Part 11 software.

Example positive results

Figure 4 *Mycoplasma* contamination (approximately 3×10^6 copies per PCR reaction)

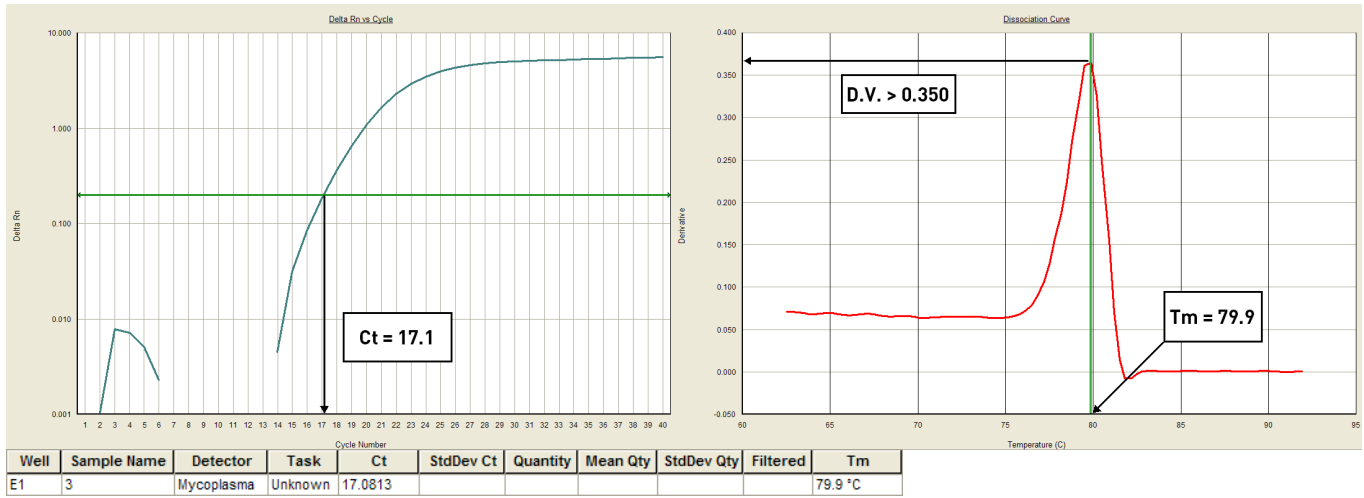


Figure 5 *Mycoplasma* contamination (approximately 2,000 copies per PCR reaction)

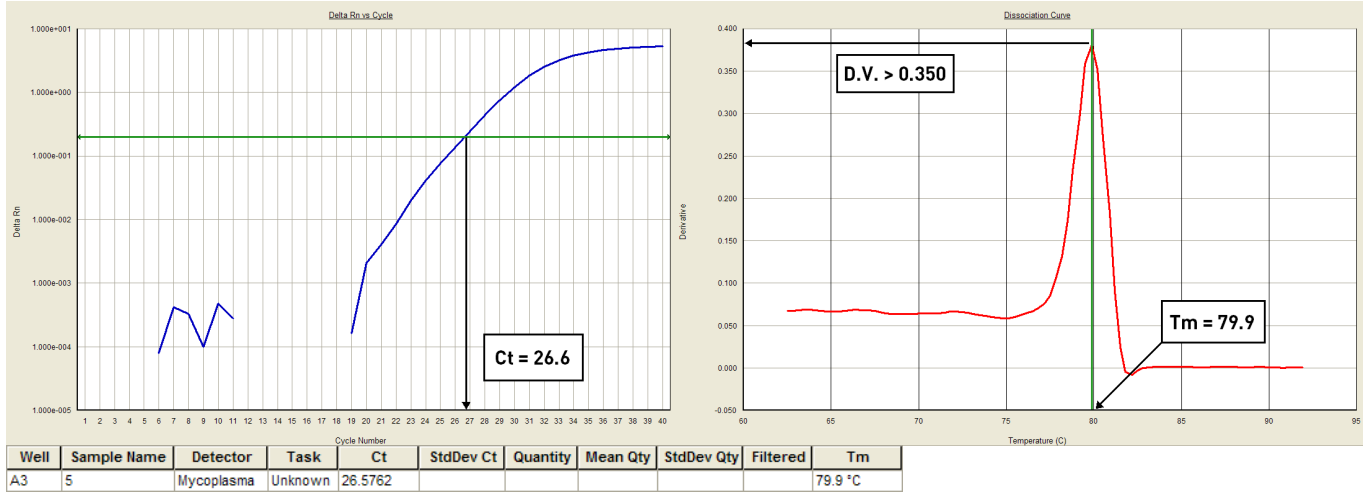
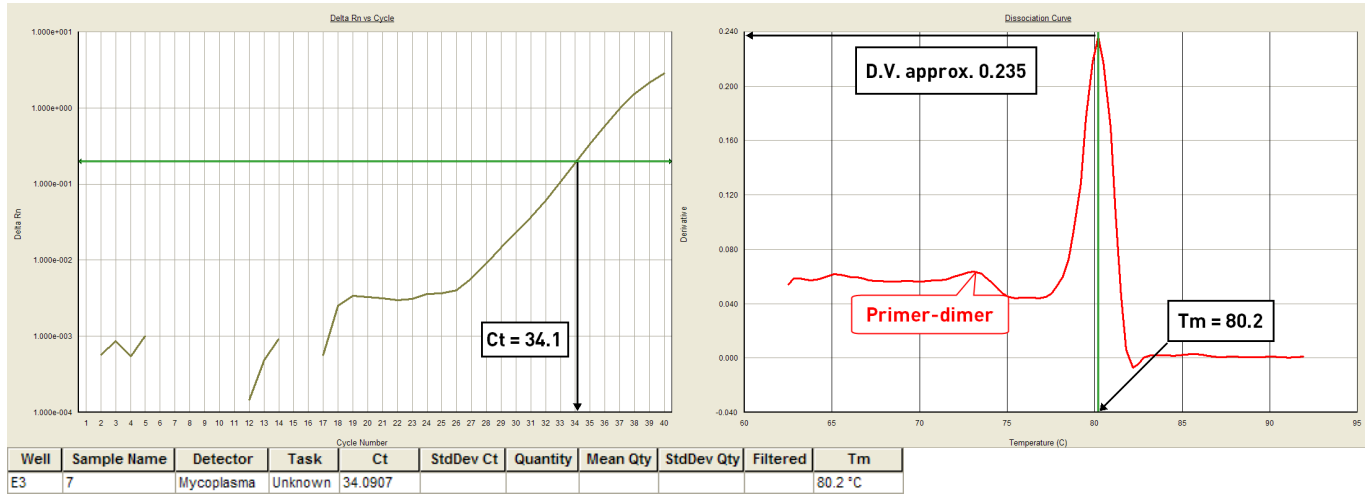


Figure 6 *Mycoplasma* contamination (less than 10 copies per PCR reaction)



Example positive control extraction results

Figure 7 Sample spiked with 2000 copies of DPC and contaminated with *Mycoplasma* (3×10^6 copies)

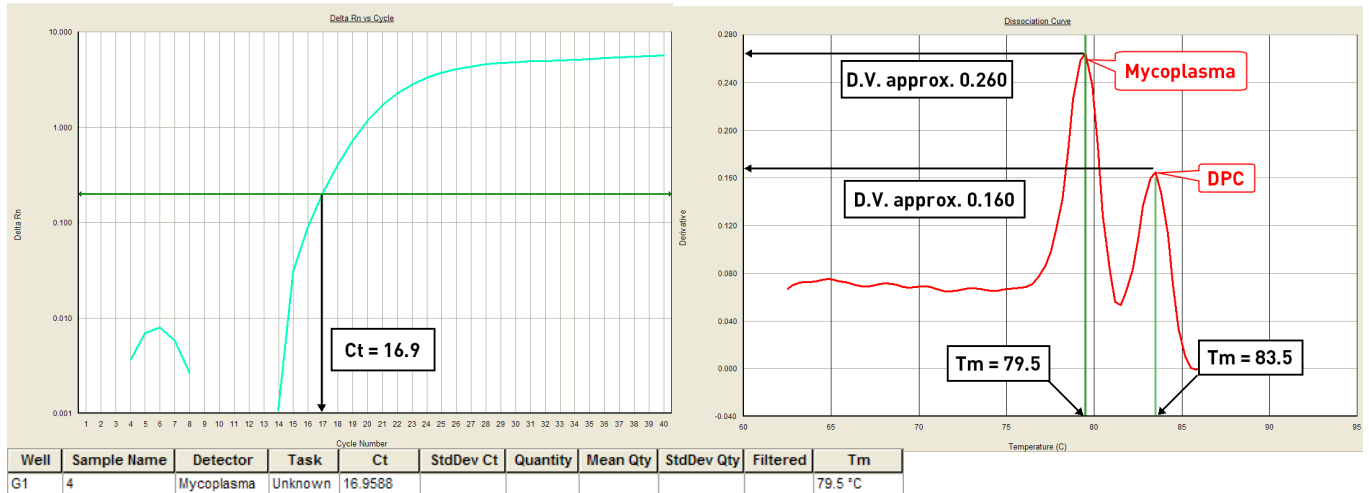


Figure 8 Sample spiked with 2000 copies of DPC and contaminated with *Mycoplasma* (approximately 2,000 copies)

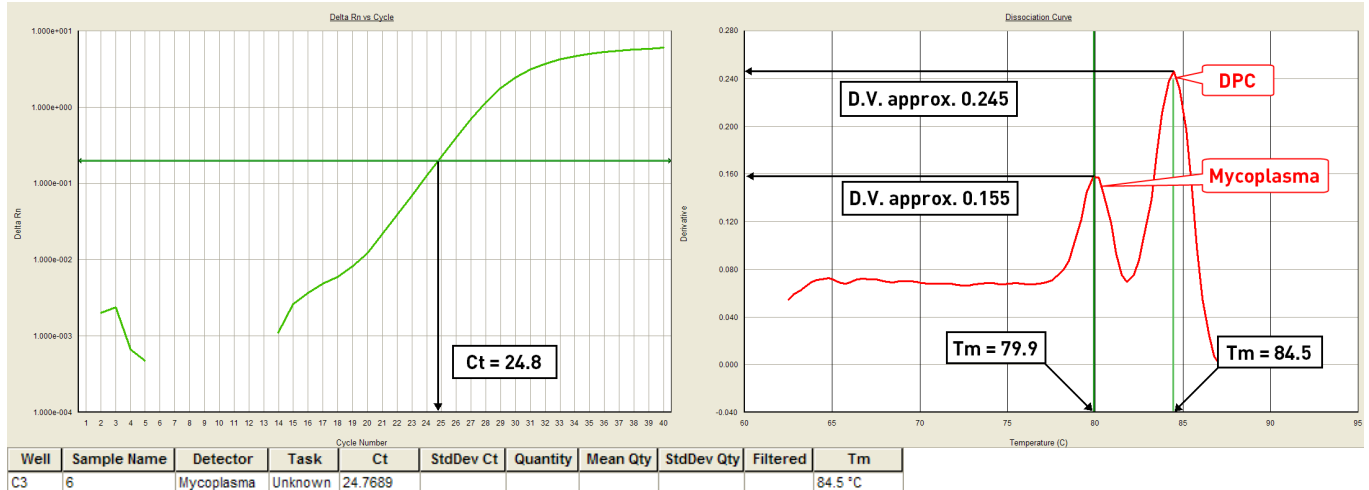
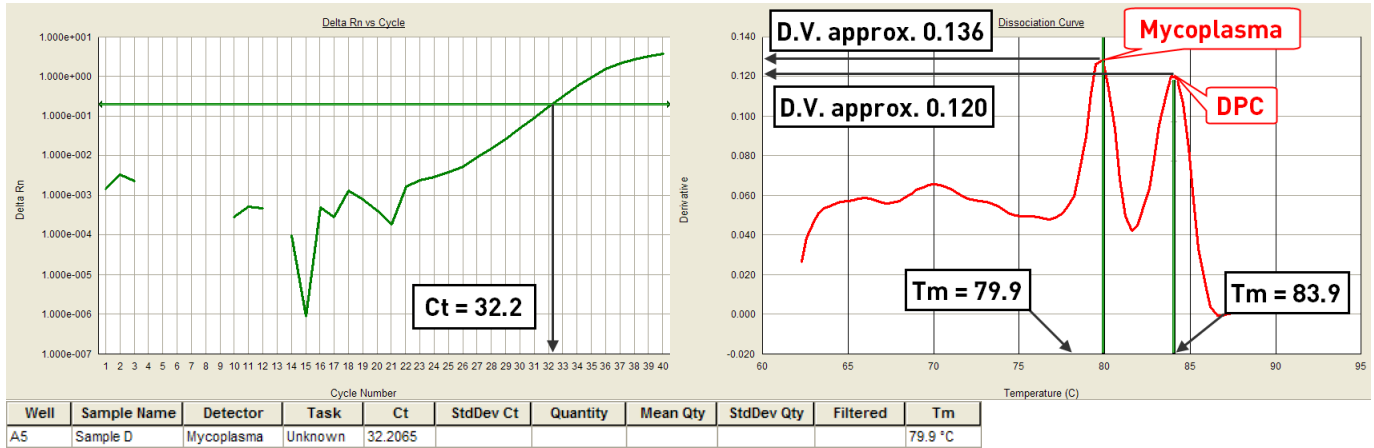


Figure 9 Sample containing 25 copies of *Mycoplasma* and 25 copies of DPC.



Example negative results

Figure 10 Negative result

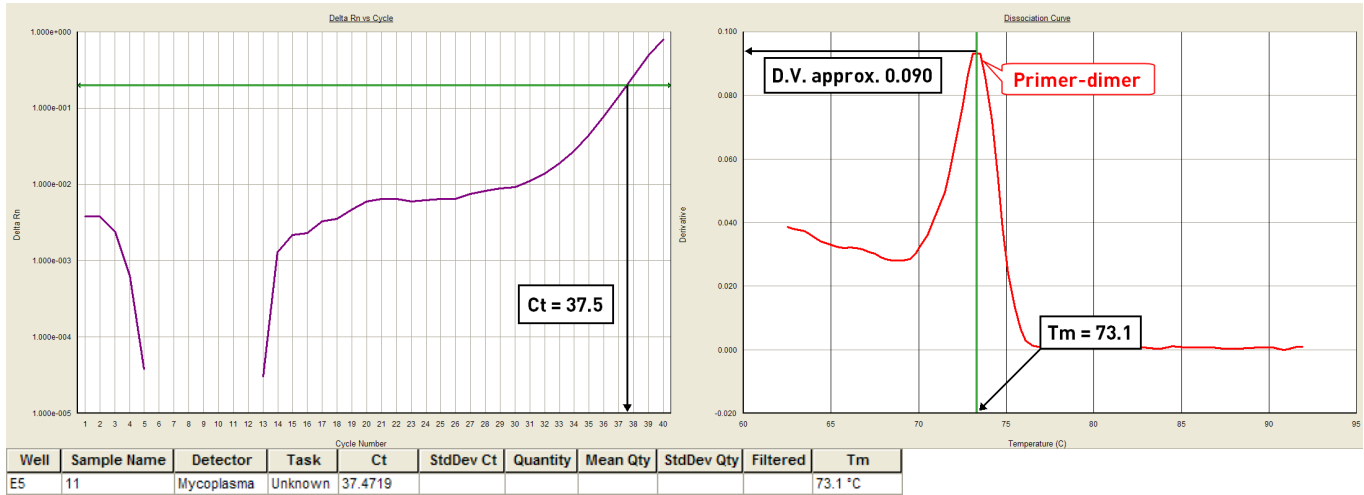
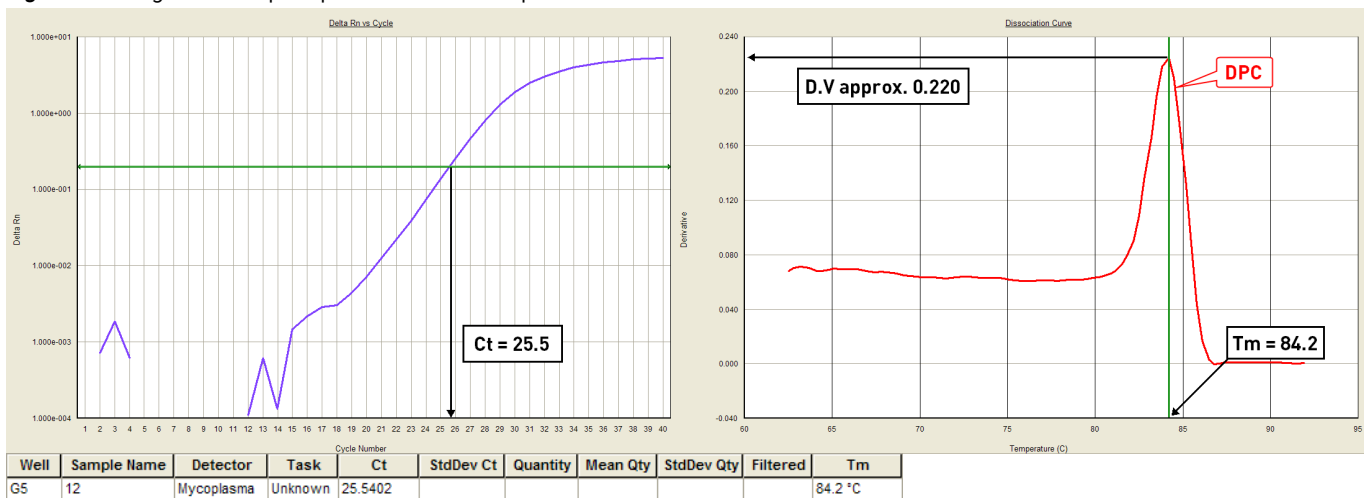



Figure 11 Negative sample spiked with 2000 copies of DPC.



Troubleshooting

AccuSEQ® software

The following table shows some common reasons for inconclusive results with AccuSEQ® software. For a complete list, click  (Help) in the toolbar at the top of the AccuSEQ® software screen. Refer to the *AccuSEQ® Real-Time PCR Detection Software Mycoplasma SEQ Experiments Getting Started Guide* (Part no. 4425587) for more data analysis information and example results.

Analysis result	Description	Possible cause	Recommended action
Presumptive positive	Based on one or more parameter, the software did not make a positive or negative call.	Low sample concentration of <i>Mycoplasma</i> .	<ul style="list-style-type: none"> • Call manually according to laboratory guidelines. or • Allow the culture to grow for an additional 24 hours, then repurify the sample and repeat the experiment using assay components that were stored correctly.
Presumptive negative			
Sample inhibits amplification	PCR inhibition shown by: <ul style="list-style-type: none"> • Negative unknown sample <i>and</i> • Inhibition control $\Delta Ct > 3$ compared to the positive control 	Inhibitors were not removed from the original sample during purification.	Repurify the sample and repeat the experiment using assay components that were stored correctly.
High background signal	High background fluorescence signal: >500,000 fluorescent standard units (FSU).	The sample block is contaminated.	Run a background calibration to identify the contaminated wells, then decontaminate the sample block. Repeat the experiment using assay components that were stored correctly.

MycoSEQ™ kits

Observation	Possible cause	Action
No positive-control or target-specific SYBR® Green dye signal is detected in positive-control wells	Inhibition of PCR	Repeat the sample preparation, then repeat the assay. If PCR remains inhibited, dilute the sample (for example, 1:10) to dilute inhibitors.
	Improper storage of Power SYBR® Green PCR Master Mix	Repeat the assay using properly stored assay components.
	Improper storage of target-specific <i>Mycoplasma</i> Real-Time PCR Primer Mix (10X)	Avoid freezing and thawing assay components. Protect Power SYBR® Green PCR Master Mix from light.
	Pipetting error (no premix solution added)	Repeat the assay. Make sure to pipet premix solution into all wells.
	Pipetting error (no positive control added)	Repeat the assay. Make sure to pipet positive control into all positive-control wells.
Target-specific signal is detected in negative-control wells	Carryover contamination	Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. If the negative control continues to show contamination, repeat the assay using a new kit. If the negative control continues to show contamination, contact Applied Biosystems Technical Support.
	High level of nonspecific product formation	Check the dissociation curve to confirm. Repeat the assay using properly stored assay components. Avoid freezing and thawing assay components. Protect Power SYBR® Green PCR Master Mix from light.
Sample is determined to be presumptive positive	Low concentrations of <i>Mycoplasma</i> in the samples	Regrow the culture for an additional 24 hours. Repurify the sample and repeat the assay using properly stored assay components.
The unknown sample is negative and the inhibition control shows a $\Delta C_T > 3$ when compared to the positive control	Inhibitors were carried over from the original sample	Repurify the sample and repeat the assay.

Background Information

Mycoplasmas are the smallest and simplest self-replicating organisms. Their genome sizes range from about 540 to 1300 kb, with a G+C content of 23 to 41 mol%. Although mycoplasmas are derived from the gram-positive branch of walled eubacteria, their evolution from these walled bacteria resulted in a substantial reduction in genome size and loss of the functions required for synthesis and maintenance of a bacterial cell wall.

Mycoplasmas are a common bacterial contaminant of cell culture samples. Infection is persistent, difficult to detect and diagnose, and very difficult to cure. Mycoplasmas vary in size from 0.2 to 0.8 μm , so they can pass through some filters used to remove bacteria. Mycoplasma in infected cell cultures can change many cell processes, including altering cell growth rate, inducing morphological changes or cell transformation, and mimicking virus infection. Cell culture in pharmaceutical production must be *Mycoplasma*-free as required by the U.S. Pharmacopoeia and FDA regulatory requirements. Therefore, there is an absolute requirement for routine, periodic testing of possible contamination of all cell cultures used in pharmaceutical manufacturing. Because mycoplasmas grow slowly (the colonies may take up to 3 weeks to develop), traditional culture methods are unacceptable for rapid high-throughput testing. The recently introduced and validated rapid bacterial testing methods that are used in this kit provide for fast *Mycoplasma* screening.



Ordering Information

Mycoseq™ *Mycoplasma* Real-Time PCR Detection Kit






Ordering

Each MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit contains reagents for 100 reactions. You can order the kit in the following configurations:

Included?	Part Number			
	4460623	4460625	4460626	4460627
Mycoseq™ <i>Mycoplasma</i> Real-Time PCR Detection Kit	Yes	Yes	Yes	Yes
Mycoseq™ Discriminatory Positive/Extraction Control	Yes	Yes	Yes	Yes
PrepSEQ® <i>Mycoplasma</i> Sample Preparation Kit	No	No	Yes	Yes
Protocol and Quick Reference Card	No	Yes	No	Yes

Kit contents and storage conditions

Note: Parts may ship separately depending on configuration and storage conditions.

Package	Part No.†	Cap color	Description	Volume	Storage	
Box 1: MycoSEQ™ <i>Mycoplasma</i> Real-Time PCR Detection Kit	Inside Box 1: <i>Mycoplasma</i> Real-Time PCR Reagents	4384774	 blue	<i>Mycoplasma</i> Real-Time PCR Primer Mix (10X), 1 tube	325 µL	-15 to -25°C‡
		362250	 white	Negative Control, 1 tube	1000 µL	
		4396882	 white	Power SYBR® Green PCR Master Mix (2X), 2 tubes	1000 µL	
	Inside Box 1: <i>Mycoplasma</i> Real-Time PCR DNA Control	4384677	 yellow	<i>Mycoplasma</i> Real-Time PCR DNA Control, 1 tube, 1000 copies/µL	700 µL	
Box 2: MycoSEQ™ Discriminatory Positive/Extraction Control	4445000	 yellow	Mycoseq™ Discriminatory Positive/Extraction Control, 1 tube, 1000 copies/µL	700 µL	-15 to -25°C	

† These part numbers are provided for identification purposes, and cannot be ordered separately.

‡ After its first use, store Box 1 at 2 to 8°C and protected from light. Excessive exposure to light may affect the Power SYBR® Green PCR Master Mix.






MycoSEQ™ Myco Scan Mycoplasma Detection Kit

Kit contents and storage conditions

The MycoSEQ™ Myco Scan Mycoplasma Detection Kit contains reagents for 25 reactions.

Note: Parts may ship separately depending on configuration and storage conditions.

MycoSEQ™ Myco Scan Mycoplasma Detection Kit (PN 4441299)

Package		Part No.	Cap color	Description	Volume	Storage
Box 1: Myco Scan <i>Mycoplasma</i> PCR Detection Kit	Inside Box 1: Myco Scan <i>Mycoplasma</i> Real-Time PCR Reagents	4441304	 orange	Myco Scan Mycoplasma Real-Time PCR Primer Mix (10X), 1 tube	100 µL	-15 to -25°C [†]
		362250	 white	Negative Control (water), 1 tube	1000 µL	
		4441312	 white	Myco Scan Power SYBR® Green PCR Master Mix (2X), 1 tube	500 µL	
	Inside Box 1: <i>Mycoplasma</i> Real-Time PCR DNA Control	4384677	 yellow	<i>Mycoplasma</i> Real-Time PCR DNA Control, 1 tube, 1000 copies/µL	700 µL	
Box 2: MycoSEQ™ Discriminatory Positive/Extraction Control		4445000	 yellow	MycoSEQ™ Discriminatory Positive/Extraction Control, 1 tube, 1000 copies/µL	700 µL	-15 to -25°C

[†] After its first use, store Box 1 at 2 to 8°C and protected from light. Excessive exposure to light may affect the Power SYBR® Green PCR Master Mix.

Materials not included in the kits

The following table includes materials required for using (but not included in) the MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit and the MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit. Unless otherwise indicated, many of the listed items are available from major laboratory suppliers (MLS).

Instruments, equipment, consumables, and reagents	
Item	Source
Instruments	
7500 Fast Real-Time PCR System	Contact your local Applied Biosystems sales office.
7500 Real-Time PCR System	
7900HT Fast Real-Time PCR System	
Equipment	
Block heater	MLS
Ice bucket	MLS
Consumables	
Disposable gloves	MLS
Aerosol-resistant pipette tips	MLS
Pipettors:	MLS
• Positive-displacement	
• Air-displacement	
• Multichannel	
MicroAmp® Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.2-mL well; for use with Applied Biosystems 7300, 7500, and 7900HT Real-Time PCR Systems	Applied Biosystems PN 4306737 Not recommended for use with the 7500 Fast system. For 7500 Fast system reactions, use PN 4346906.
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.1-mL well; for use with Applied Biosystems 7500 Fast Real-Time PCR System	Applied Biosystems PN 4346906
MicroAmp® Optical 96-Well Reaction Plate with Barcode and Optical Adhesive Films, 100 plates with covers; for use with 7300 and 7500 Real-Time PCR Systems	Applied Biosystems PN 4314320
MicroAmp® Optical 8-Cap Strip, 300 strips	Applied Biosystems PN 4323032
MicroAmp® Optical Adhesive Film Kit, 20 covers, 1 applicator, 1 optical cover compression pad	Applied Biosystems PN 4313663
MicroAmp® Optical Adhesive Film, 25 covers	Applied Biosystems PN 4360954
Reagents	
DNase-free, sterile-filtered water	MLS

B

Appendix B Ordering Information *Materials not included in the kits*

Good PCR Practices

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule. Follow the guidelines below to prevent contamination and nonspecific amplification.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
 - Sample preparation
 - PCR setup
 - PCR amplification
 - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution.

IMPORTANT! To avoid false positives due to cross-contamination:

- Prepare and close all negative-control and unknown sample tubes before pipetting the positive control.
 - Do not open tubes after amplification.
 - Use different sets of pipettors when pipetting negative-control, unknown, and positive-control samples.
-

Plate layout suggestions

- For each plate row, dispense in sequence from left to right the: negative controls, unknown samples, inhibition controls, and positive controls (at the end of the row or column).
- Place positive controls in one of the outer columns.
- If possible, separate all samples from each other by at least one well; if space is limited, place at least one well between unknown samples and controls.
- Be aware that caps come in strips of 8 or 12.

Kit Specificity

Product description

The MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit and the MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit detect *Mycoplasma* species simply, reliably, and rapidly. To detect the presence of these microorganisms, the assay uses the polymerase chain reaction (PCR) to amplify a target unique to a wide variety of mycoplasmas.

Sensitivity

The sensitivity of the PCR using this kit is 1 to 10 copies of the target DNA per reaction. Sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used. The sample preparation procedure in the *PrepSEQ® Sample Preparation Kits User Guide* (PN 4465957) allows you to detect:

- 4 to 10 CFU/mL of *Mycoplasma* from 10 mL of cell culture
- or
- 4 CFU/mL of *Mycoplasma* from 1 mL of media

Kit specificity

The MycoSEQ™ *Mycoplasma* Detection Kits: MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit, and MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit can detect more than 90 different *Mycoplasma* species, including *Acholeplasma laidlawii* and *Spiroplasma citri*. The kit does not detect other genera or cell-line DNA.

Inclusivity – detectable species

The kit procedure in this protocol is designed to detect over 90 species, including the 14 shown below in the first table. For a complete list of species, contact Applied Biosystems.

Species	Strain/source
<i>Acholeplasma laidlawii</i>	ATCC 23206D
<i>Mycoplasma arginini</i>	ATCC 23838D
<i>Mycoplasma fermentans</i>	ATCC 19989D
<i>Mycoplasma gallisepticum</i>	ATCC 15302
<i>Mycoplasma genitalium</i>	ATCC 33530D
<i>Mycoplasma hominis</i>	ATCC 23114D
<i>Mycoplasma hyorhinis</i>	ATCC 17981D
<i>Mycoplasma hyponeumoniae</i>	ATCC 25095
<i>Mycoplasma orale</i>	ATCC 23714D
<i>Mycoplasma pirum</i>	ATCC 25960D
<i>Mycoplasma pneumoniae</i>	ATCC 15531D
<i>Mycoplasma salivarium</i>	ATCC 23064D
<i>Mycoplasma sinoviae</i>	ATCC 25204
<i>Spiroplasma citri</i>	ATCC 27556D

Exclusivity – undetectable organisms

Organism	Strain/source
<i>Bacillus cereus</i>	ATCC 10876
<i>Bacillus subtilis</i>	ATCC 6051
<i>Campylobacter jejuni</i>	ATCC 29428
<i>Citrobacter freundii</i>	6879
<i>Clostridium perfringens</i>	ATCC 12915
<i>Enterobacter aerogenes</i>	Q87
<i>Enterobacter sakazaki</i>	ATCC 51329
<i>Enterococcus faecalis</i>	ATCC 29212
<i>Escherichia coli</i> O157:H7	43888
<i>Klebsiella oxytoca</i>	ATCC 43165
<i>Lactobacillus bulgaris</i>	ATCC 11842
<i>Listeria ivanovii</i>	ATCC 19119
<i>Listeria monocytogenes</i>	ATCC 7644
<i>Pseudomonas aeruginosa</i>	ATCC 27853
<i>Pseudomonas aeruginosa</i>	ATCC 17423
<i>Shigella</i>	Sfla 395
<i>Shigella</i>	SFL 153
<i>Shigella dysenteriae</i>	ATCC 13313
<i>Shigella dysenteriae</i>	ESCL7-JHH
<i>Staphylococcus aureus</i>	ATCC 43300
<i>Staphylococcus aureus aureus</i>	PE491
<i>Streptococcus faecalis</i>	ATCC 9790
<i>Vibrio cholerae</i>	O36
<i>Yersinia enterocolitica</i>	ATCC 9610
Cat	Novagen, catalog # 69235-3
Cow	Novagen, catalog # 69238-3
Chicken	Novagen, catalog # 69233-3
Chimpanzee	Bios, Inc. [†]
CHO	ATCC CCL-61
HeLa	ATCC CCL-2
Horse	Pel-Freez Biologicals, catalog # 39339-5
Orangutang	Bios, Inc. [†]
Pig	Novagen, catalog # 69230-3
Rabbit	Pel-Freez Biologicals, catalog # 31130-1
Rat	Novagen, catalog # 69238-3
Sheep	Novagen, catalog # 69231-3

† No longer available

Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
 - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
-



Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
 - Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
 - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
 - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
 - Handle chemical wastes in a fume hood.
 - Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
 - After emptying a waste container, seal it with the cap provided.
 - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
 - Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
 - **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
-

Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: www.cdc.gov/biosafety
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: www.cdc.gov

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/



Documentation and Support

Related documentation

The following related documents are shipped with the system:

Real-time PCR system	Document	PN	
All real-time PCR systems	<i>MycoSEQ™ Mycoplasma Detection Kits Quick Reference Card: MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit, MycoSEQ™ Myco Scan Mycoplasma Detection Kit</i>	4393471	Provides brief, concise instructions on using the MycoSEQ™ Mycoplasma Detection Kits.
	<i>MycoSEQ™ Mycoplasma Detection Kits User Guide</i>	4465874	Describes the MycoSEQ™ Mycoplasma Detection Kits and provides information on preparing, running, and troubleshooting Mycoplasma detection.
	<i>ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit Quick Reference Card</i>	4445236	Provides brief, concise instructions on using the ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit.
	<i>ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit Protocol</i>	4445235	Describes the ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit and provides information on preparing, running, and troubleshooting MMV detection.
	<i>PrepSEQ® Sample Preparation Kits Quick Reference Card</i>	4406304	Provides brief, concise instructions on using the PrepSEQ® Sample Preparation Kits.
	<i>PrepSEQ® Sample Preparation Kits User Guide</i>	4465957	Describes the PrepSEQ® Sample Preparation Kits and provides information on preparing, running, and troubleshooting sample preparation.
	<i>PrepSEQ® Nucleic Acid Extraction Kit Quick Reference Card</i>	406303	Provides brief, concise instructions on using the PrepSEQ® Nucleic Acid Extraction Kit.
	<i>PrepSEQ® Nucleic Acid Extraction Kit Protocol</i>	4400739	Describes the PrepSEQ® Nucleic Acid Extraction Kit and provides information on preparing, running, and troubleshooting nucleic acid extractions.
	<i>Introduction to TaqMan® and SYBR® Green Chemistries for Real-Time PCR Protocol</i>	4407003	Describes the TaqMan® and SYBR® Green Chemistries for Real-Time PCR and provides information on preparing, running, and troubleshooting PCR.

Real-time PCR system	Document	PN	
Analysis software	<i>AccuSEQ® Real-Time PCR Detection Software Mycoplasma SEQ Experiments Getting Started Guide</i>	4425587	Provides brief, step-by-step procedures for Mycoplasma detection. It is designed to help you quickly learn to use the AccuSEQ® Real-Time PCR Detection Software for Mycoplasma SEQ Experiments.
7900 Fast system	<i>Applied Biosystems 7900 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i>	4364014	Provides brief, step-by-step procedures for absolute quantitation using a standard curve. It is designed to help you quickly learn to use the Applied Biosystems 7900 Fast Real-Time PCR System.
7300, 7500, and 7500 Fast systems	<i>Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide</i>	4347825	Provides brief, step-by-step procedures for absolute quantitation using a standard curve. It is designed to help you quickly learn to use the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System.

For information on new assays and updated product documentation, go to www.microseq.com.

Portable document format (PDF) versions of this guide and the documents listed above are available at www.appliedbiosystems.com

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.appliedbiosystems.com/sds

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Glossary

amplification	The process of making copies of and thereby increasing the amount of a specific DNA sequence.
polymerase chain reaction (PCR)	Technology used to increase the amount of a DNA sequence.
Power SYBR [®] Green PCR Master Mix	The master mix used to prepare the premix solution. It contains the DNA polymerase enzyme that initiates PCR in the presence of the necessary primers and DNA sample. It also contains SYBR [®] Green I dye, which binds to double-stranded (ds) DNA, thus providing a fluorescence signal that indicates the amount of dsDNA product generated during PCR.
negative control	A reaction solution that lacks a target sequence. A negative control monitors for contamination (unexpected amplification in the absence of a target) and reagent integrity. At least one negative control is required per run.
inhibition control	A reaction solution that includes the Power SYBR [®] Green PCR Master Mix, the unknown sample, and the positive control (<i>Mycoplasma</i> Real-Time PCR DNA control or MycoSEQ [™] Discriminatory Positive/Extraction Control). An inhibition control monitors for inhibitors in the unknown sample (inhibition in the presence of a positive target).
<i>Mycoplasma</i> Real-Time PCR DNA Control	A specially designed plasmid DNA used as the positive control whose amplification mimics the expected amplification of a target. Target signal that is not detected in a positive-control well indicates a pipetting error or a problem with amplification. At least one positive control is required per run.
MycoSEQ [™] Discriminatory Positive/Extraction Control	<p>A multi-purpose control designed to:</p> <ul style="list-style-type: none">• Evaluate DNA extraction efficiency during sample preparation• Discriminate between a true positive-sample and an accidental cross-contamination with the Positive Control• Detect inhibition during the Real-Time PCR assay <p>The control is designed for use with the PrepSEQ[®] <i>Mycoplasma</i> Nucleic Acid Extraction Kit and the MycoSEQ[®] <i>Mycoplasma</i> Real-Time PCR Detection Kit.</p>
primer	A segment of DNA that is complementary to the target DNA sequence and is needed to start amplification.
target	The bacteria being tested.
unknown sample	A DNA sample from media, cell culture, or other source that you are testing for the presence of <i>Mycoplasma</i> .

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