Applied Biosystems

MycoSEQ™ Mycoplasma Detection Kits:

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

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Part Number 4393111 Rev. D
06/2010

Contents

About this protocol

This protocol provides:

- Background information about the detection of Mycoplasma species
- A list of materials and equipment that can be used with the MycoSEQ™ Mycoplasma Detection Kits: MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit, 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

Safety information

Note: For general safety information, see this Preface and Appendix E, “Safety” on page 25. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

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. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see Appendix E.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

How to use this guide

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. 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, accurate chemistry kit use, or safe use of a chemical.

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**.

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

MycoSEQ™ Mycoplasma Detection Kits: MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit, MycoSEQ™ Myco Scan Mycoplasma Detection Kit

Kit workflow

Workflow for using the MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit and MycoSEQ™ Myco Scan Mycoplasma Detection Kit:

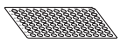
IMPORTANT! For information on how to avoid PCR contamination, see Appendix C on page 19.

Prepare the sample
page 2

Prepare for PCR
Prepare the plate document
page 2

Prepare the kit reagents and premix solution
page 5

Prepare the PCR reactions
page 6



Perform PCR
page 7

Analyze the results
page 8

Prepare the sample

Refer to the PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit Protocol (PN 4401253) for details on sample preparation.

Prepare for PCR

Prepare the plate document

1. When you set up the plate document, 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. 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*.

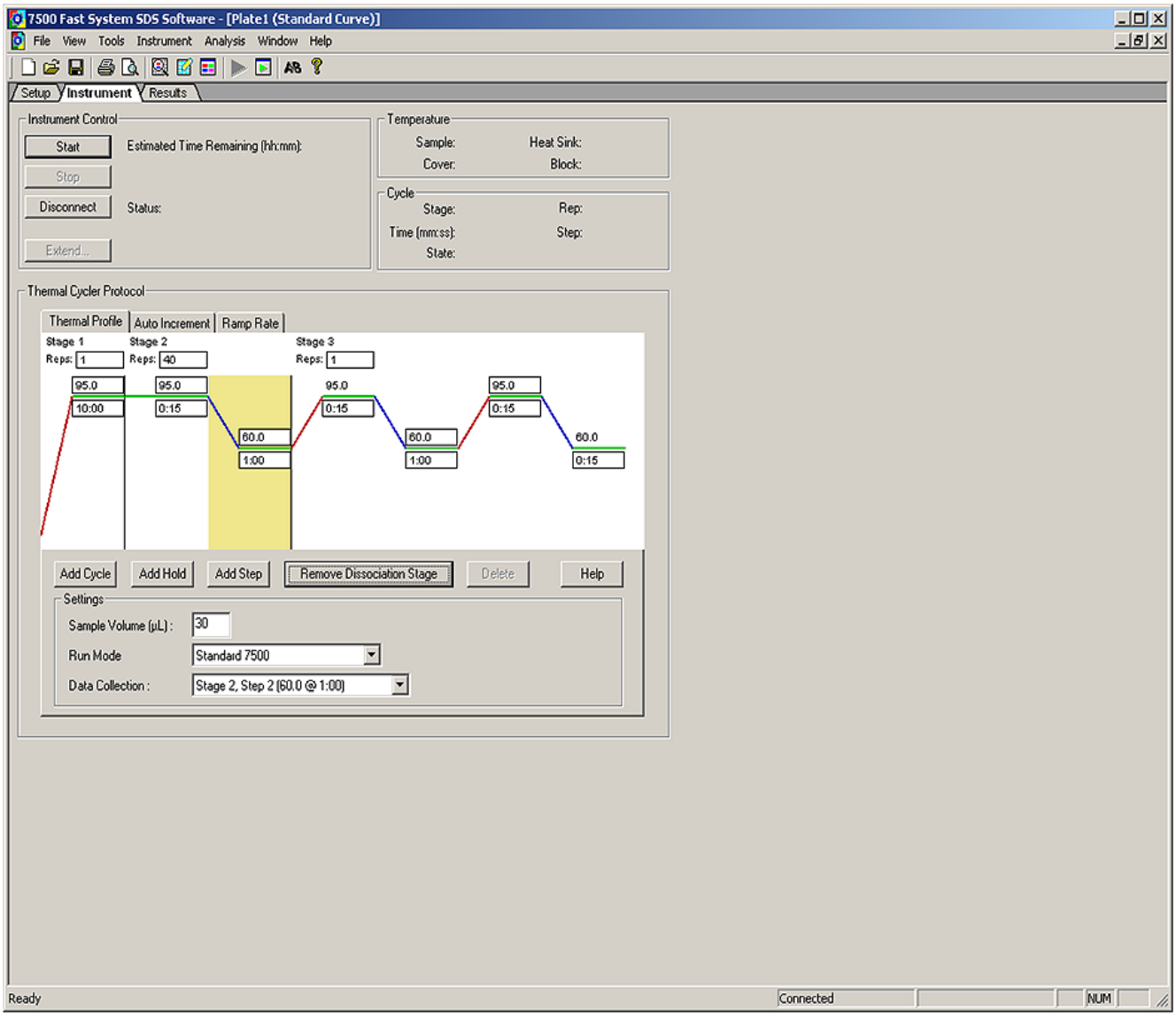
Step	AmpliTaq Gold® enzyme activation	PCR		Dissociation‡§#			
	HOLD	Cycle (40 cycles)		Melt			
		Denature	Anneal/extend				
Temp	95 °C	95 °C	60 °C	95 °C	60 °C	95 °C	60 °C
Time	10 min	15 sec	1 min	15 sec	1 min	15 sec	15 sec

‡ 7500 and 7500 Fast Systems: from the Instrument tab, click **Add Dissociation Stage** (see figure on page 3).

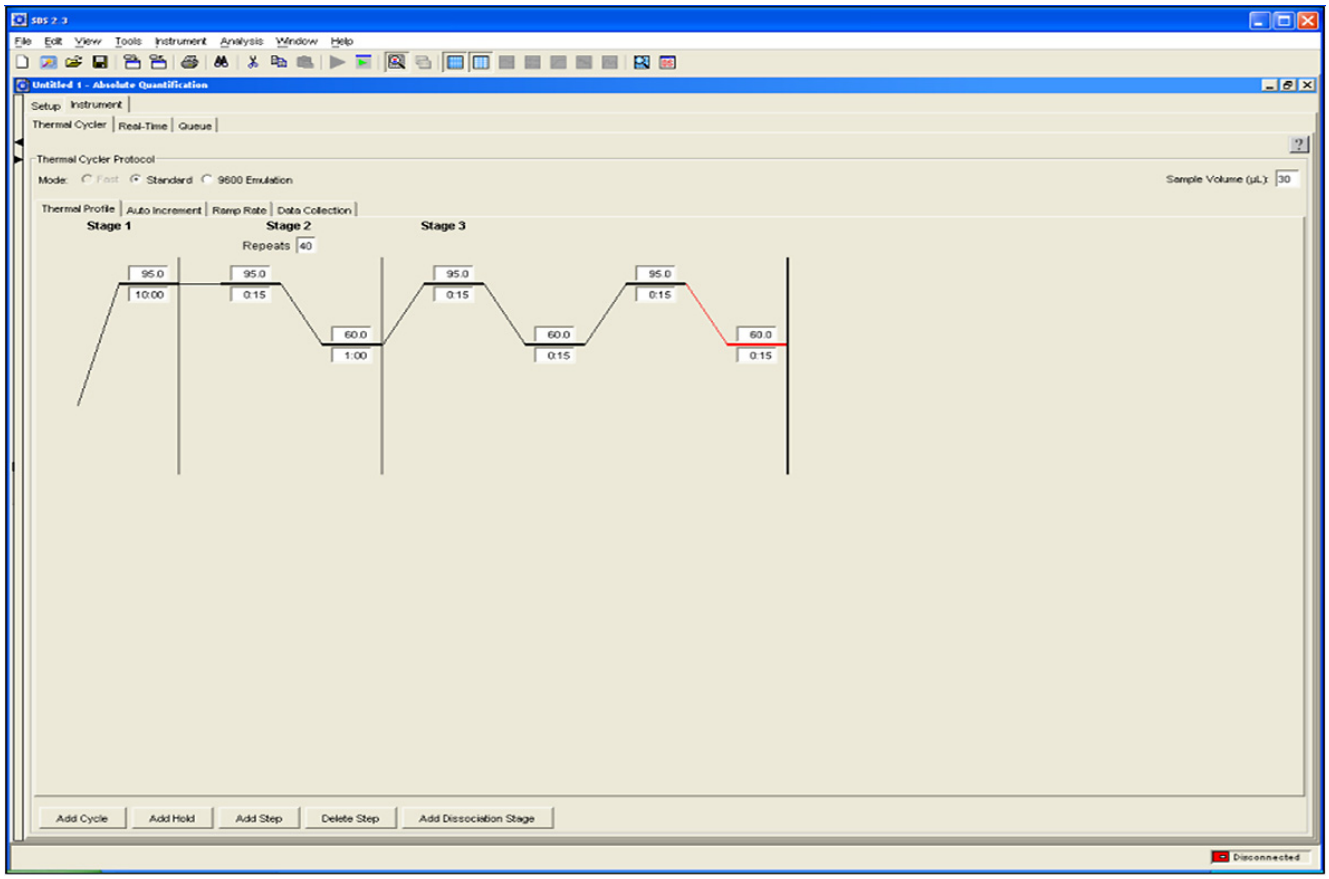
§ (Optional) 7900HT Fast Systems: from the Instrument tab, click **Add Dissociation Stage**, then click **Add Step** (to set the four temperatures required during the Dissociation Stage; see figure on page 4).

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

- Set Sample Volume to **30** µL.
- For the 7500 Fast system, SYBR® Green I dye can be used with Run Mode set to **Standard 7500**.
- For the 7900HT Fast system, SYBR® Green I dye can be used with Run Mode set to **Standard**.



The instrument tab for 7500 Fast Time Real-Time PCR platform with SDS 1.4 21 CFR Part 11 software. The run mode is set to Standard 7500.



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. Label a microcentrifuge tube for the premix solution, and for each sample and control reaction.

4. Prepare the Premix Solution according to the following table.

IMPORTANT! Use a separate pipette tip for the Power SYBR® Green PCR Master Mix and the Mycoplasma Real-Time PCR Primer Mix.

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
Mycoplasma Real-Time PCR Primer Mix (10X) or Myco Scan Mycoplasma 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.

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

Prepare the PCR reactions

1. Pipette 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	• Add 18 µL of Premix Solution • Add 12 µL of Negative Control (water)
Your unknown sample reaction	• Add 18 µL of Premix Solution • Add 2 to 10 µL of unknown sample • Adjust the final reaction volume to 30 µL with Negative Control (water)
Inhibition-control reaction	• Add 18 µL of Premix Solution • Add 2 to 10 µL of unknown sample • Add 2 µL of Mycoplasma Real-Time PCR DNA Control (positive control) • Adjust the final reaction volume to 30 µL with Negative Control (water)
Positive-control reaction	• Add 18 µL of Premix Solution • Add 2 µL of Mycoplasma Real-Time PCR DNA Control (positive control) • Add 10 µL of Negative Control (water)

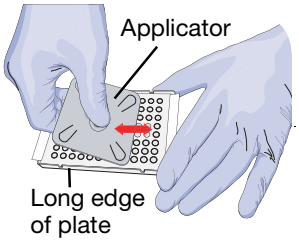
2. Dispense 18 µL of Premix Solution into each well to be used, gently pipetting at the bottom of the well. For the:
   - 7500 Fast system – Dispense into a Fast optical 96-well plate (PN 4346906).
   - 7500 and 7900HT Fast (standard block) systems – Dispense into a standard optical 96-well plate (PN 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 "Plate layout suggestions" on page 20 for more information.

   Pipetting guidelines:
   - Use at least one negative and one positive control per run.
   - Mix each sample very gently by placing the pipette tip at the bottom of the tube and pipetting up and down to minimize aerosol formation and cross-contamination.
   - Use a new tip for each well, even when aliquoting the same solution.
   - Keep all reactions on ice.

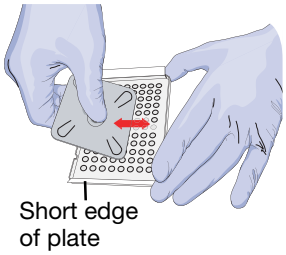
Seal the plates

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.



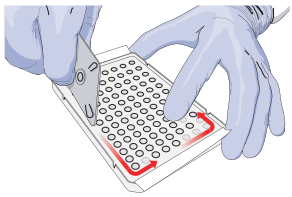
IMPORTANT! Apply significant downward pressure on the applicator to completely seal the wells. Pressure is required to activate the adhesive on the optical cover.

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 × g for 20 seconds using a centrifuge with a plate adapter.

IMPORTANT! Make sure 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 you created on page 2.

2. Load the reaction plate into the real-time PCR system.

3. Start the run.

Analyze the results

For instructions on how to analyze your results, refer to the user guide of your real-time PCR instrument. General guidelines:

- View the amplification plots for the entire plate.
- Set the baseline and threshold values. For all reactions, use default Analysis Settings:
  - Select **Manual Baseline**.
  - Set Start (cycle) to **3**.
  - Set End (cycle) to **15**.
  - Set Threshold to **0.2**.
- Examine the SYBR Green dye signal in all wells. Evaluate and record the:
  - C_T values from amplification plots
  - Derivative values from dissociation curves
- Use the following table as a basic guide for evaluating the results:

SYBR Green dye signal	Derivative target T_m 75 to 85 °C and derivative >0.1	Derivative no-target T_m <75 °C	Result
Present, C_T <36	Present	Absent	Positive
Present, C_T <36	Present	Present	Positive
Present, C_T <36	Absent	Present	Presumptive negative
Present, but C_T >36	Present	Absent	Presumptive positive
Present, but C_T >36	Present	Present	Presumptive positive
Present, but C_T >36	Absent	Absent	Presumptive negative
Present, but C_T >36	Absent	Present	Presumptive negative
Absent	Absent	Absent	Negative
Absent	Absent	Present	Negative

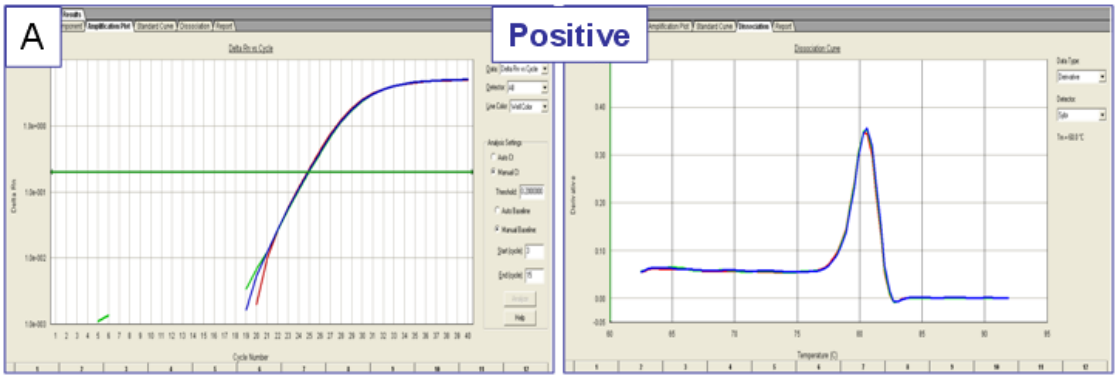
IMPORTANT! You must repurify a presumptive negative sample, then repeat the assay. Allow the culture to grow an additional 24 hours before repeating the assay.

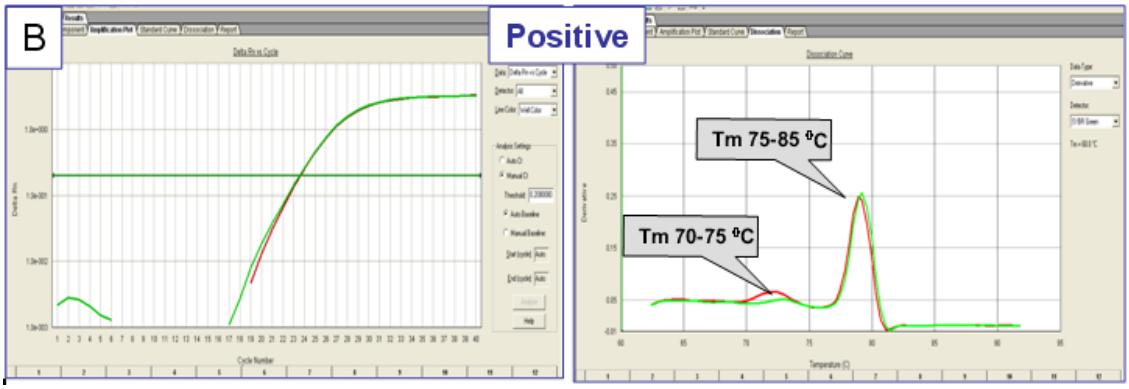
Interpretation

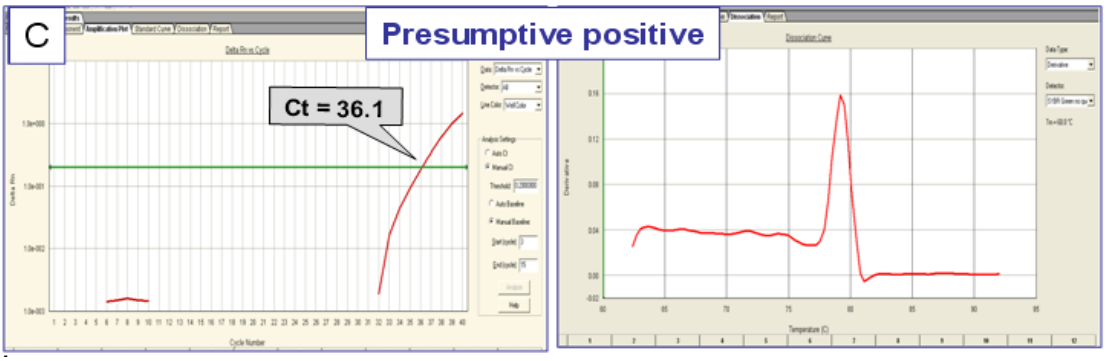
To call a run positive, the inhibition control must be positive.

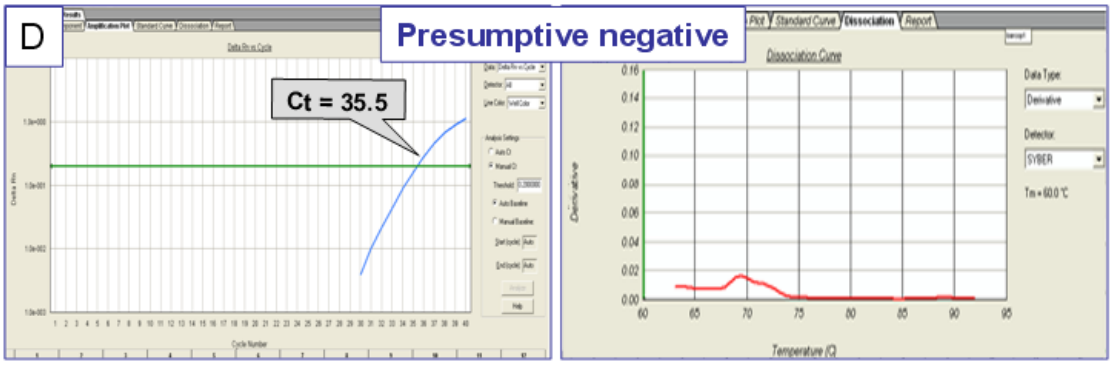
The difference in C_T between a positive-control reaction and the inhibition-control reaction should be less than 3 C_T (ΔC_T <3). If the unknown sample is negative, and the inhibition control shows a ΔC_T >3 when compared to the positive control, repurify the sample and repeat the assay.

Note: The following examples show positive, presumptive positive, and presumptive negative results.


A
Positive


B
Positive
Tm 75-85 ºC
Tm 70-75 ºC


C
Presumptive positive
Ct = 36.1


D
Presumptive negative
Ct = 35.5
Dissociation Curve
Data Type
Derivative
Detector
SYBER
Tm = 60.0 °C
Temperature (C)

Troubleshooting

Observation	Possible cause	Action
No positive-control or target-specific SYBR Green dye signal is detectedin 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 Mycoplasma Real-Time PCR Primer Mix (10×)	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 pipette premix solution into all wells.
	Pipetting error (no positive control added)	Repeat the assay. Make sure to pipette 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 Mycoplasma 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 ΔC_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

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.

Description of target microorganisms

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. They vary in size from 0.2 to 0.8 µm and, therefore, 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.

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™ Mycoplasma Nucleic Acid Extraction Kits Protocol* (PN 4401253) 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, 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. For more information, refer to Appendix D on page 21.

Definitions of terms

This protocol uses the following terms:

- **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. 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 (*Mycoplasma* Real-Time PCR DNA control). Monitors for inhibitors in the unknown sample (inhibition in the presence of a positive target).
- **Mycoplasma 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.

- **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 Mycoplasma.

Ordering Information

Kit contents

The MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit contains reagents for 100 reactions. Components and their storage conditions are shown below.

MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit (PN 4384772)

Package	Part number	Cap color	Description	Volume (µL)	Storage
Box 1: *Mycoplasma* Real-Time PCR Reagents (PN 4399461)	4384774	blue	*Mycoplasma* Real-Time PCR Primer Mix (10×), 1 tube	325	–15 to –25 °C‡
	362250	white	Negative Control, 1 tube	1000	
	4396882	white	Power SYBR® Green PCR Master Mix (2×), 2 tubes	1000	
Box 2: *Mycoplasma* Real-Time PCR Control (PN 4399364)	4384677	clear	*Mycoplasma* Real-Time PCR DNA Control, 1 tube, 1000 copies/µL	400	–15 to –25 °C; minimize freeze-thaw cycles

‡ 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.

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

The MycoSEQ™ Myco Scan Mycoplasma Detection Kit contains reagents for 25 reactions. Components and their storage conditions are shown below.

MycoSEQ™ Myco Scan Mycoplasma Detection Kit (PN 4441299)

Package	Part number	Cap color	Description	Volume (µL)	Storage
Box 1: Myco Scan Mycoplasma Real-Time PCR Reagents (PN 4441305)	4441304	orange	Myco Scan Mycoplasma Real-Time PCR Primer Mix(10×), 1 tube	100	–15 to –25 °C‡
	362250	white	Negative Control (water), 1 tube	1000	
	4441312	white	Myco Scan Power SYBR® Green PCR Master Mix (2×), 1 tube	500	
Box 2: *Mycoplasma* Real-Time PCR Control (PN 4399364)	4384677	clear	*Mycoplasma* Real-Time PCR DNA Control, 1 tube, 1000 copies/µL	400	–15 to –25 °C; minimize freeze-thaw cycles

‡ 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.

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

Materials not included in the kit

Table 1 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: • Positive-displacement • Air-displacement • Multichannel	MLS
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

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 limiting, place at least one well between unknown samples and controls.
- Be aware that caps come in strips of 8 or 12.

Kit Specificity

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

Exclusivity – undetectable organisms

Organism	Strain/source
Bacillus cereus	ATCC 10876
Bacillus subtilis	ATCC 6051
Campylobacter jejuni	ATCC 29428

Organism	**Strain/source** *(continued)*
Citrobacter freundii	6879
Clostridium perfringens	ATCC 12915
Enterobacter aerogenes	Q87
Enterobacter sakazaki	ATCC 51329
Enterococcus faecalis	ATCC 29212
Escherichia coli O157:H7	43888
Klebsiella oxytoca	ATCC 43165
Lactobacillus bulgaris	ATCC 11842
Listeria ivanovii	ATCC 19119
Listeria monocytogenes	ATCC 7644
Pseudomonas aeruginosa	ATCC 27853
Pseudomonas aeruginosa	ATCC 17423
Shigella	Sfla 395
Shigella	SFL 153
Shigella dysenteriae	ATCC 13313
Shigella dysenteriae	ESCL7-JHH
Staphylococcus aureus	ATCC 43300
Staphylococcus aureus aureus	PE491
Streptococcus faecalis	ATCC 9790
Vibrio cholerae	O36
Yersinia enterocolitica	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

Organism	**Strain/source** *(continued)*
Rat	Novagen, catalog # 69238-3
Sheep	Novagen, catalog # 69231-3

‡ No longer available

Appendix E

Safety

This appendix covers:

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs” on page 27.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you select

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

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets (MSDSs) and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument 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

General biohazard

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:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **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**

Chemical alerts

General alerts for all chemicals

Avoid contact with (skin, eyes, and/or clothing). Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Related documentation

For additional documentation, see "How to obtain support" on page vii.

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

Real-time PCR system	Document	PN
All real-time PCR systems	*MycoSEQ™ Mycoplasma Detection Kits Quick Reference Card: MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit, MycoSEQ™ Myco Scan Mycoplasma Detection Kit*	4393471
	PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit Protocol	4401253
	PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit Quick Reference Card	4406304
	PrepSEQ™ Nucleic Acid Extraction Kit Protocol	4400739
	PrepSEQ™ Nucleic Acid Extraction Kit Quick Reference Card	4406303
	Introduction to TaqMan® and SYBR® Green Chemistries for Real-Time PCR Protocol	4407003
7900 Fast system	*Applied Biosystems 7900 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide*	4364014
7300, 7500, and 7500 Fast systems	*Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide*	4347825

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

Note: To open the documentation available from the Applied Biosystems web site, use the Adobe® Acrobat® Reader® software available at **www.adobe.com**

Part Number 4393111 Rev. D 06/2010

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