



# TaqMan<sup>®</sup> *Francisella tularensis* Detection Kit

Catalog Number 4382490 Publication Number 4384224 Revision C



For testing of Food and Environmental samples only.

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## About this guide

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

### **Revision history**

Revision	Date	Description
С	December 2014	<ul> <li>Updated recommended DNA isolation methods section for general use.</li> <li>Updated instructions for use of recent models of real-time PCR instruments.</li> <li>Updated protocol organization and some number formatting (e.g., temperature and time ranges) to align with the current style guide.</li> <li>Updated user guide template with associated updates to the covers, legal, document support, and safety sections.</li> </ul>
В	August 2010	Update of the PCR Patent
А	April 2007	New document



## **Product information**

### **Product description**

TaqMan <sup>®</sup> Pathogen Detection Kits provide a simple, reliable, and rapid meth the detection of contaminants in food and environmental samples. The assay polymerase chain reaction (PCR) to amplify unique microorganism-specific E target sequences and TaqMan <sup>®</sup> probes to detect the amplified sequences.	
	The TaqMan <sup>®</sup> Pathogen Detection Kits include an internal positive control (IPC) to monitor for PCR inhibition. The IPC also demonstrates whether or not PCR reagents are working and amplifying properly. This IPC eliminates the need for a positive control, thus reducing the risk of cross-contamination in unknown samples. The detection of the IPC provides greater confidence in negative samples.
	<b>Note:</b> We recommend that users perform validation with their unique sample matrices/types to determine appropriate analysis settings (ISO 22174, 2005).
Dual single-plex assays	The TaqMan <sup>®</sup> <i>Francisella tularensis</i> Detection Kit includes two Target Assay Mixes, one targeting <i>tul4</i> and the other targeting <i>fopA</i> . Both Target Assay Mixes include IPC. For each sample, prepare two single-plex PCRs using one Target Assay Mix per PCR.

### Kit contents and storage

Sufficient reagents are supplied for 50 reactions (30- $\mu L$  reaction volume) for each Target Assay Mix.

Cap color	Volume	Storage <sup>[1]</sup>
Purple	300 µL	Upon receipt, below –18°C.
Green	300 µL	Protect from light. <sup>[3]</sup> Minimize freeze-thaw cycles.
White	1000 µL	5±3°C
Red	2 × 750 μL	5±3°C
	Purple Green White	Purple     300 μL       Green     300 μL       White     1000 μL

<sup>[1]</sup> Refer to the product label for expiration date.

[2] Contains primers and probes for amplification and detection of target (FAM<sup>™</sup>-dye labeled probe) and IPC (VIC<sup>®</sup>-dye labeled probe)

<sup>[3]</sup> Excessive exposure to light may affect the fluorescent probes.

**Note:** Kit components may ship separately depending on configuration and storage conditions.

### Required materials not included with the kit

Unless otherwise indicated, all materials are available from Life Technologies (**www.lifetechnologies.com**). MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instrument and equipment	
<ul> <li>One of the following real-time PCR systems:</li> <li>Applied Biosystems<sup>®</sup> 7500 Fast Real-Time PCR System</li> <li>Applied Biosystems<sup>®</sup> StepOnePlus<sup>™</sup> Real-Time PCR System</li> <li>Applied Biosystems<sup>®</sup> StepOne<sup>™</sup> Real-Time PCR System</li> </ul>	Contact your sales representative.
Benchtop microcentrifuge	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
Precision Plate Holder for 7500 Fast System, as app	ropriate for your experiment
For plates	Cat. no. 4359652
For 0.1-mL tube strips	Cat. no. 4403809
Optical reaction plates and covers, <i>or</i> optical PCR tu your instrument	bes and caps, as appropriate for
Plates for use with the StepOne <sup>™</sup> Real-Time PCR Syst	em:
MicroAmp <sup>®</sup> Fast Optical 48-Well Reaction Plate	Cat. no. 4375816
MicroAmp <sup>®</sup> 48-Well Optical Adhesive Film	Cat. no. 4375928
Plates for use with the StepOnePlus <sup>™</sup> Real-Time PCR PCR System:	System or 7500 Fast Real-Time
MicroAmp <sup>®</sup> Fast Optical 96-Well Reaction Plate	Cat. no. 4346907 (no barcode) Cat. no. 4366932 (with barcode)
MicroAmp <sup>®</sup> Optical Adhesive Film	Cat. no. 4311971
Tubes for use with all specified real-time PCR system	15:
MicroAmp <sup>®</sup> Fast 8-Tube Strip	Cat. no. 4358293
MicroAmp <sup>®</sup> Optical 8-Cap Strips	Cat. no. 4323032
Other consumables	



Item	Source	
Aerosol-resistant pipette tips	MLS	
Disposable gloves	MLS	
MicroAmp <sup>®</sup> Splash-free Support Base	Cat. no. 4312063	
Sterile microcentrifuge tubes	MLS	
Reagent		
Nuclease-Free Water (not DEPC-Treated)	Cat. no. AM9938	

### Additional materials for enrichment and DNA isolation

Spin columns

Spin columns

#### General Depending on your sample type, enrichment (that is, growing the specific pathogen from the sample matrix) may be the first step in using the TaqMan<sup>®</sup> Francisella recommendations tularensis Detection Kit . You will need enrichment reagents and a protocol for enrichment appropriate for the matrix and pathogen of interest. We recommend that you validate your matrices with the most current FDA BAM enrichment methods. Enrichment time for this PCR-based pathogen detection kit can be reduced significantly from standard microbiology enrichment protocols. This kit has been validated using our internal design and development standards; however, it has not been validated for any specific application or use. We recommend that you validate these kits for your particular application or use. For most food or environmental sample types, we suggest using one of the following Suggested DNA sample preparation options: isolation methods **Nucleic acid isolation** Kit Cat. no. workflow PrepSEQ<sup>®</sup> Nucleic Acid Extraction Kit for Food Automated, magnetic 4428176, bead-based and Environmental Testing 4480466 PrepSEQ<sup>®</sup> Rapid Spin Sample Preparation Kit Spin columns 4426714 with Proteinase K PrepSEQ<sup>®</sup> Rapid Spin Sample Preparation Kit -

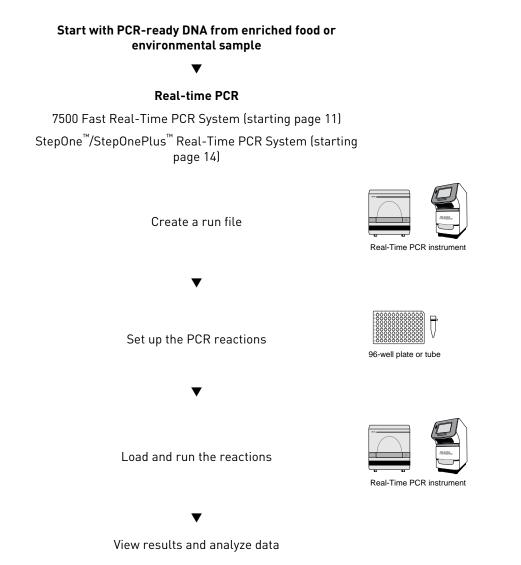
Extra Clean with Proteinase K

PrepSEQ<sup>®</sup> Rapid Spin Sample Preparation Kit

4426715

4407760

#### Workflow



### Important procedural guidelines

**Note:** All TaqMan<sup>®</sup> Pathogen Detection Kits run with a single, standard PCR protocol, allowing them to be combined on the same plate. This feature allows screening for multiple pathogens in the same PCR run.

- Run both Target Assay Mixes (*tul4* and *fopA*) in parallel for specific identification and confirmation of the presence of *Francisella tularensis*.
- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use new tips when pipetting the Master Mix, Target Assay Mix, Target Premix Solution, and each negative control and sample.
- Use at least one no-template control per target organism tested.
- During PCR set up, mix very gently with the pipette tip at the bottom of the tube, to minimize aerosol formation and cross-contamination.

- When mixing samples by pipetting up and down, avoid creating bubbles.
- To maintain strip orientation when transferring tubes to the instrument tray, mark or label one end of the strip cap (but not over a cap).
- Do not cut the 8-tube strips. For 8-tube strips with seven or fewer reactions, leave the empty tubes as blanks so that each strip contains a full set of 8 tubes. The empty, capped tubes evenly distribute the clamping load that is applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.
- Follow the recommendations in Chapter 2, "TaqMan<sup>®</sup> Francisella tularensis Detection Kit using the 7500 Fast Real-Time PCR System" or Chapter 3, "TaqMan<sup>®</sup> Francisella tularensis Detection Kit using the StepOne<sup>™</sup> or StepOnePlus<sup>™</sup> Real-Time PCR System" to prepare reagents for plates and tube strips. The volumes are sufficient for one plate or one strip plus overage. To calculate volumes for other sample numbers, refer to the per-reaction volume and add 10% overage.
- Follow the recommendations in "How to seal plates for the PCR run" on page 22 or in "How to seal tubes for the PCR run" on page 23 for sealing the reaction vessels.
- Follow the recommendations in "Good laboratory practices for PCR and RT-PCR" on page 21, including "Plate layout suggestions" on page 21.



## TaqMan<sup>®</sup> *Francisella tularensis* Detection Kit using the 7500 Fast Real-Time PCR System

#### Create a run file

For detailed instructions on setup and programming the Applied Biosystems<sup>®</sup> 7500 Fast Real-Time PCR Instrument, refer to the guide accompanying your instrument or to the 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide (Pub. no. 4347825).

- **1.** Select **Standard Curve (Absolute Quantification)** from the Assay drop-down list.
- 2. Select FAM and VIC dye detectors with the Quencher Dye set to None or Non-Fluorescent.
- 3. Associate dyes with each reaction.
- 4. Name each reaction as desired.
- **5.** Set thermal cycling conditions for the 7500 Fast Real-Time PCR Instrument according to the following table.

Settings	Stage 1 Enzyme activation		ge 2 CR
Number of	1 (Hold)	45 cy	/cles
cycles		Denature	Anneal/extend
Temperature	95°C	95°C	60°C
Time	20 sec	3 sec	30 sec

- **6.** Set Sample Volume to **30**  $\mu$ L.
- 7. Select Fast Run Mode.



**Chapter 2** TaqMan<sup>®</sup> *Francisella tularensis* Detection Kit using the 7500 Fast Real-Time PCR System *Set up the PCR reactions* 

### Set up the PCR reactions

- **1.** Thaw all reagents completely.
- **2.** Prepare a sufficient volume of each Target Premix Solution according to the following tables. Mix by pipetting up and down gently.

 Table 1
 Target Premix Solution tul4

Component	Volume per well or tube	Volume per 96–well plate	Volume per 8-tube strip
2X Fast PCR Master Mix	15 µL	1.5 mL	135 µL
10X Target Assay Mix, <i>tul4</i>	3 µL	300 µL	27 µL
Total Target Premix Solution <i>tul4</i>	18 µL	1.8 mL	162 μL

 Table 2
 Target Premix Solution fopA

Component	Volume per well or tube	Volume per 96-well plate	Volume per 8-tube strip
2X Fast PCR Master Mix	15 µL	1.5 mL	135 µL
10X Target Assay Mix, <i>fopA</i>	3 µL	300 µL	27 µL
Total Target Premix Solution <i>fopA</i>	18 µL	1.8 mL	162 μL

- **3.** Transfer 18 µL of Target Premix Solution *tul4* to the bottom of every other sample well or tube.
- **4.** Transfer 18  $\mu$ L of Target Premix Solution *fopA* to the bottom of the remaining empty wells or tubes.
- **5.** Add 12  $\mu$ L of sample or control to each pair of wells or tubes. Gently mix the solution by pipetting up and down.
- **6.** Close the tubes or apply an optical cover to the plate.
- **7.** (*Optional*) Spin down the reagents to ensure that they are at the bottom of the wells or tubes.

### Load and run the reactions

1. Load the reactions into the instrument with the appropriate 7500 Fast Precision Plate Holder on the block.

Reaction vessel	Loading method	
96-well plates	Place the plate on the sample block.	
8-tube strips	Load the strips vertically in the center of the block.	
	<b>Note:</b> If columns 1 and 12 are not used, fill them with em strips. This will balance the block to avoid damage of tube	

2. Close the instrument loading block and start the run.

### View results and analyze data

Refer to the instrument user guide for instructions on how to analyze data and view your results.

- 1. View the amplification plots for all samples.
- **2.** Set the baseline and threshold values.
- **3.** Check each sample for a FAM<sup>™</sup> dye (target-specific) signal and a VIC<sup>®</sup> dye (IPC) signal.

The following table provides a basic guide for interpreting the results:

FAM <sup>™</sup> dye signal (target)	VIC <sup>®</sup> dye signal (IPC)	Result
+	+, -	Positive
-	+	Negative
_	_	See Appendix A, "Troubleshooting"



## TaqMan<sup>®</sup> *Francisella tularensis* Detection Kit using the StepOne<sup>™</sup> or StepOnePlus<sup>™</sup> Real-Time PCR System

### Create a run file

The following instructions apply to use of the StepOne<sup>TM</sup> software on the StepOne<sup>TM</sup> or StepOnePlus<sup>TM</sup> Real-Time PCR System. For detailed instructions on setup and programming the instrument, refer to the *StepOne<sup>TM</sup>* and *StepOnePlus<sup>TM</sup> Real-Time PCR System User Guide* (Pub. no. 4379704).

1. Go to File > New experiment, and select Advanced Setup.

For	Select	
Instrument	StepOne <sup>™</sup> Instrument – (48 Wells)	
	or	
	StepOnePlus <sup>™</sup> Instrument – (96 Wells)	
Type of experiment	Quantitation – Standard Curve	
Type of reagents	TaqMan <sup>®</sup> Reagents	
Ramp speed	Fast	

2. In the Experimental Properties page, select the following settings:

**3.** In the Plate Setup page, do the following:

Tab	Create or select
Define Targets and Samples	<ul> <li>Create 2 targets with quenchers set to None.</li> <li>Select FAM<sup>™</sup> and VIC<sup>®</sup> dyes as the reporters.<sup>[1]</sup></li> </ul>
Assign Targets and Samples	Associate the $FAM^{^{\mathrm{M}}}$ and $VIC^{^{\otimes}}$ dyes with each reaction.

<sup>[1]</sup> FAM<sup>™</sup> dye is used to detect the targets, and VIC<sup>®</sup> dye is used to detect the IPC.

**4.** In the Run Method page, set the thermal cycling conditions according to the following table:

Settings	Stage 1 Enzyme activation		ge 2 CR
Number of	1 (Hold)	45 c <u>y</u>	ycles
cycles	(10tu)	Denature	Anneal/extend
Temperature	95°C	95°C	60°C
Time	20 sec	3 sec	30 sec

- 5. Set Reaction Volume to  $30 \ \mu$ L.
- 6. Under File, save the run file as a *.eds* document in the appropriate folder.

#### Set up the PCR reactions

- **1.** Thaw all reagents completely.
- **2.** Prepare a sufficient volume of each Target Premix Solution according to the following tables. Mix by pipetting up and down gently.

Table 3	Target Premix Solution tul4
---------	-----------------------------

Component	Volume per well or tube	Volume per 48–well plate	Volume per 96–well plate	Volume per 8-tube strip
2X Fast PCR Master Mix	15 µL	750 μL	1.5 mL	135 µL
10X Target Assay Mix, <i>tul4</i>	3 µL	150 μL	300 µL	27 µL
Total Target Premix Solution <i>tul4</i>	18 µL	900 µL	1.8 mL	162 µL

Table 4 Target Premix Solution fopA

Component	Volume per well or tube	Volume per 48–well plate	Volume per 96–well plate	Volume per 8-tube strip
2X Fast PCR Master Mix	15 µL	750 μL	1.5 mL	135 µL
10X Target Assay Mix, <i>fopA</i>	3 µL	150 μL	300 µL	27 µL
Total Target Premix Solution <i>fopA</i>	18 µL	900 µL	1.8 mL	162 µL

- **3.** Transfer 18  $\mu$ L of Target Premix Solution *tul4* to the bottom of every other sample well or tube.
- **4.** Transfer 18  $\mu$ L of Target Premix Solution *fopA* to the bottom of the remaining empty wells or tubes.

TaqMan<sup>®</sup> Francisella tularensis Detection Kit User Guide

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- 5. Add 12  $\mu$ L of sample or control to each pair of wells or tubes. Gently mix the solution by pipetting up and down.
- **6.** Close the tubes or apply an optical cover to the plate.
- **7.** (*Optional*) Spin down the reagents to ensure that they are at the bottom of the wells or tubes.

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### Load and run the reactions

Instrument	Reaction vessel	Loading method
StepOne <sup>™</sup> Real-Time PCR	48–well plate	Place the plate on the sample block.
System	8-well strips	Load the strips horizontally.
		<b>Note:</b> For example, in Row C, load an 8-tube strip across columns 1 through 8. A minimum of one 8-tube strip is recommended. It is not necessary to balance the tube strips on the tray.
Step0nePlus <sup>™</sup> Real-Time	96–well plate	Place the plate on the sample block.
PCR System	8-well strips	Load the strips vertically.
		<b>Note:</b> The minimum recommended load is two 8-tube strips (16 tubes), which should be placed in adjacent columns, for example in columns 1 and 2. It is not necessary to balance the tube strips on the tray.

1. Load the reactions into the instrument.

**2.** Open the appropriate run file document and start the run.



**IMPORTANT!** To avoid false positives due to amplified material in your work area, do not open tubes after amplification.

### View results and analyze data

Refer to the instrument user guide for instructions on how to analyze data and view your results.

- 1. View the amplification plots for all samples.
- **2.** Set the baseline and threshold values.
- **3.** Check each sample for a FAM<sup>™</sup> dye (target-specific) signal and a VIC<sup>®</sup> dye (IPC) signal.

The following table provides a basic guide for interpreting the results:

FAM <sup>™</sup> dye signal (target)	VIC <sup>®</sup> dye signal (IPC)	Result
+	+, -	Positive
-	+	Negative
-	_	See Appendix A, "Troubleshooting"



## Troubleshooting

Observation	Possible cause	Recommended action
In unknown wells, no IPC or target-specific signal is detected.	Inhibition of PCR occurred.	Dilute the sample 1:5 with Nuclease-Free Water, to dilute PCR inhibitors, and repeat the assay. If PCR remains inhibited, repeat the sample preparation.
		Alternatively, use a bacterial genomic DNA purification kit (major laboratory supplier) to remove inhibitors.
	Master Mix not stored     properly.	Repeat the assay using properly stored assay components.
	10X Target Assay Mix not	Avoid freezing and thawing assay components.
	stored properly.	Protect the Target Assay Mix from light.
	Pipetting error (no premix solution added).	Repeat the assay. Make sure to pipette premix solution into all wells.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA exists in samples, resulting in preferential amplification of the target- specific DNA.	No action is required. The result is considered positive.
In negative control wells, no IPC signal is detected, but a target-specific signal is	Carryover contamination caused target signal in negative control wells.	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.
detected.	<ul> <li>Additionally, no IPC signal in negative control wells can be caused by:</li> <li>A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA.</li> <li>A problem occurred with</li> </ul>	To determine whether IPC amplification is a problem, examine unknown wells for an IPC signal. If an IPC signal is present, IPC amplification is not a problem.
In negative control wells, target-specific signal is detected.	IPC amplification. Carryover contamination occurred.	<ol> <li>Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.</li> <li>If the negative control continues to show contamination, repeat the assay using a new kit.</li> <li>If the negative control continues to show contamination, contact Technical Support.</li> </ol>

Observation	Possible cause	Recommended action
Replicate results for a sample are inconsistent.	All replicate wells for a sample do not have the same result.	If more than two replicates yield the same result (for example, 2 of 3 replicates are negative, but 1 replicate is positive), refer to your laboratory protocol to determine whether to repeat the assay using fresh samples and reagents.
		If only 2 replicates were run and the results are not consistent, repeat the assay using fresh samples and reagents.

### Supplemental information

### Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified 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 and reaction setup.
    - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap<sup>™</sup> Solutions (Cat. no. AM9890).

For additional information, refer to ISO 22174 (2005).

### Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps for PCR tubes come in strips of 8 or 12.

### How to seal plates for the PCR run

**IMPORTANT!** Apply significant downward pressure on the applicator in all steps to form a complete seal on top of the wells. Pressure is required to activate the adhesive on the optical cover.

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.



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



- **3.** Rub the end of the applicator horizontally and vertically between all wells.
- **4.** Rub the end of the applicator around all outside edges of the plate using small back and forth motions to form a complete seal around the outside wells.



#### How to seal tubes for the PCR run

**IMPORTANT!** Apply significant downward pressure on the sealing tool in all steps to form a complete seal on top of the tubes.

- 1. Place strip caps on the tubes.
- 2. Seal the tubes using one of the following methods:
  - If you are using the rolling capping tool:



- a. Roll the capping tool across all strips of caps on the short edge, then the long edge, of the plate.
- b. Roll the capping tool around all outer rows of strips of caps.
- If you are using the rocking capping tool:



- a. Slip your fingers through the handle with the holes in the tool facing down.
- b. Place the holes in the tool over the first eight caps in a row.
- c. Rock the tool back and forth a few times to seal the caps.
- d. Repeat for remaining caps in the row, then for all remaining rows.

## 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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

• U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:



www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

• World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:

www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

### **Documentation and support**

#### **Customer and technical support**

Visit **www.lifetechnologies.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

#### Food Safety support

Website: www.lifetechnologies.com/foodsafety

Support email: foodsafety@lifetech.com

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit **www.lifetechnologies.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

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### References

ISO. 2005. Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions. Reference number 22174:2005.

U.S. Food and Drug Administration, Bacteriological Analytical Manual (BAM); go to **www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm**. Accessed 13 Jan. 2014.

