USER GUIDE

applied biosystems® by life technologies™

TaqMan® *E. coli* O157:H7 Detection Kit

Catalog Number 4366100

Publication Number 4366569

Revision D

For testing of Food and Environmental samples only.

life technologies™

For testing of Food and Environmental samples only.

The information in this guide is subject to change without notice.

DISCLAIMER

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

LIMITED USE LABEL LICENSE No. 492: Environmental Testing, Quality Control/Quality Assurance Testing, Food and Agricultural Testing

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) for environmental testing, quality control/quality assurance testing, food and agricultural testing, including reporting results of purchaser's activities in environmental testing, quality control/quality assurance testing, food and agricultural testing for a fee or other commercial consideration. No other right is hereby granted expressly, by implication, or by estoppel. This product is for environmental testing, quality control/ quality assurance testing, food and agricultural testing and research purposes only.

The purchase of this product does not grant the purchaser any additional rights, including (without limitation) the right to transfer or resell the product in any form or the right to use the product as a therapeutic agent or diagnostics test component. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Trademarks

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

©2014 Thermo Fisher Scientific Inc. All rights reserved.

Contents

About this guide

Revision history

Revision	Date	Description
D	March 2014	• Updated recommended enrichment and DNA isolation methods sections for general use. • Updated instructions for use of recent models of real-time PCR instruments. • Updated protocol organization and some number formatting (e.g., temperature and time ranges) to align with the current style guide. • Updated user guide template with associated updates to the covers, legal, document support, and safety sections.
C	June 2010	Baseline for revision history.

1 Product information

IMPORTANT! Before using the products described in this guide, read and understand the information in the "Safety" appendix in this document.

Product description

TaqMan® Pathogen Detection Kits provide a simple, reliable, and rapid method for the detection of contaminants in food and environmental samples. The assay uses the polymerase chain reaction (PCR) to amplify unique microorganism-specific DNA target sequences and TaqMan® probes to detect the amplified sequences.

The TaqMan® 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 minimizes the need for a positive control, and reduces the risk of cross-contamination in unknown samples. The detection of the IPC provides greater confidence in negative samples.

Note: We recommend that the user perform validation with their unique sample matrices/types to determine appropriate analysis settings (ISO 22174, 2005). Life Technologies offers fee-based method validation and verification services; contact **foodsafety@lifetech.com** for more information.

Kit contents and storage

Sufficient reagents are supplied for 100 reactions (30-µL reaction volume).

Table 1 The TaqMan® *E. coli* O157:H7 Detection Kit (Cat. no. 4366100).

Components	Cap color	Volume	Storage[1]
10X *E. coli* O157:H7 Target Assay Mix, 1 tube[2]	Green	300 µL	Upon receipt, below –18°C. Protect from light.[3] Minimize freeze-thaw cycles.
Negative control, 1 tube	White	1000 µL	5±3°C

Components	Cap color	Volume	Storage[1]
2X Environmental Master Mix, 2 tubes	Yellow/ Gold	2 × 750 µL	5±3°C

[1] Refer to the product label for expiration date.

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

[3] 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. MLS: major laboratory supplier.

Item	Source
Instrument and equipment	
One of the following real-time PCR systems: • Applied Biosystems® 7500 Fast Real-Time PCR System • Applied Biosystems® StepOnePlus™ Real-Time PCR System • Applied Biosystems® StepOne™ Real-Time PCR System	Contact your local Life Technologies representative.
Benchtop microcentrifuge	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel	MLS
Precision Plate Holder for 7500 Fast System, as appropriate for your experiment	
For plates	Cat. no. 4403809
For 0.1-mL tube strips	Cat. no. 4359652
Optical reaction plates and covers, *or* optical PCR tubes and caps, as appropriate for your instrument	
Plates for use with the StepOne™ Real-Time PCR System:	
MicroAmp® Fast Optical 48-Well Reaction Plate	Cat. no. 4375816
MicroAmp® 48-Well Optical Adhesive Film	Cat. no. 4375928

Item	Source
Plates for use with the StepOnePlus™ Real-Time PCR System or 7500 Fast Real-Time PCR System:	
MicroAmp® Fast Optical 96-Well Reaction Plate	Cat. no. 4346907 (no barcode) Cat. no. 4366932 (with barcode)
MicroAmp® Optical Adhesive Film	Cat. no. 4311971
Tubes for use with all specified real-time PCR systems:	
MicroAmp® Fast 8-Tube Strip	Cat. no. 4358293
MicroAmp® Optical 8-Cap Strips	Cat. no. 4323032
Other consumables	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
MicroAmp® Splash-free Support Base	Cat. no. 4312063
Sterile microcentrifuge tubes	MLS
Reagent	
Nuclease-free Water	Cat. no. AM9938

Additional materials for enrichment and DNA isolation

General recommendations for enrichment

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® *E. coli* O157:H7 Detection Kit. You will need enrichment reagents and a protocol appropriate for the matrix and pathogen of interest.

We recommend that you validate your matrices with the most current USDA FSIS MLG or FDA BAM enrichment methods. For most food types, enrichment time for this PCR-based pathogen detection kit can be reduced significantly from standard microbiology enrichment protocols. We recommend that you optimize the time needed to enrich your specific sample.

Recommended DNA isolation methods

For most food or environmental sample types, we recommend using one of the following sample preparation options:

Nucleic acid isolation workflow	Kit	Cat. no.
Automated, magnetic bead-based	PrepSEQ® Nucleic Acid Extraction Kit for Food and Environmental Testing	4480466, 4428176
Spin columns	PrepSEQ® Rapid Spin Sample Preparation Kit with Proteinase K	4426714

Nucleic acid isolation workflow	Kit	Cat. no.
Spin columns	PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K	4426715
Spin columns	PrepSEQ® Rapid Spin Sample Preparation Kit	4407760
Direct lysis	Lysis Buffer, FS and Proteinase K, FS	4480724 and 4480715

Workflow

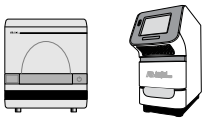
Start with PCR-ready DNA from enriched food or environmental sample

▼

Real-time PCR

▼

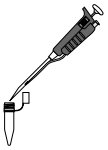
Create a run file



Real-Time PCR instrument

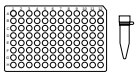
▼

Prepare the Target Premix Solution



▼

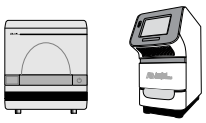
Set up the PCR reactions



96-well plate or tube

▼

Load and run the reactions



Real-Time PCR instrument

▼

View results and data analysis

Important procedural guidelines

Note: All TaqMan® 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.

- Use new tips when pipetting the Environmental Master Mix, Target Assay Mix, Target Premix Solution, and each unknown 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.
- 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).
- For 8-tube strips with seven or fewer reactions, add additional empty tubes as needed 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 "Good laboratory practices for PCR and RT-PCR" on page 22, including "Plate layout suggestions" on page 23.

2 TaqMan® *E. coli* O157:H7 Detection Kit using the 7500 Fast Real-Time PCR System

Create a run file

For detailed instructions on setup and programming the Applied Biosystems® 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	Stage 2 PCR	
Number of cycles	1 (Hold)	40 cycles	
		Denature	Anneal/extend
Temperature	95°C	95°C	60°C
Time	10 min	15 sec	45 sec

6. Set Sample Volume to **30 µL**.
7. Select **Standard** Run Mode.

Set up the PCR reactions

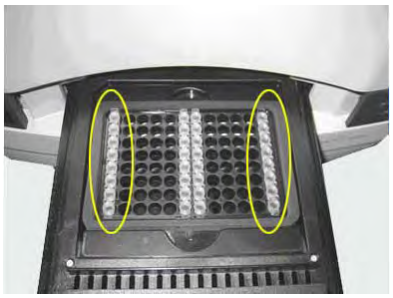
1. Thaw all reagents completely.
2. Prepare the Target Premix Solution: combine the following components for the number of reactions (positive and negative controls) plus 10% overage, and pipet up and down gently to mix.

Component	Vol. for one 30-µL reaction
2X Environmental Master Mix	15.0 µL
10X Target Assay Mix	3.0 µL
Total volume	**18.0 µL**

3. Transfer 18 µL of Target Premix Solution into each well to be used, gently pipetting at the bottom of the well.
4. Add 12 µL of unknown samples and negative controls to each well as appropriate. Gently pipet up and down to mix the solution.
5. Close the tubes or apply an optical cover to the plate. See "How to seal plates for the PCR run" on page 23 or "How to seal tubes for the PCR run" on page 24.
6. Make sure that the reagents are in the bottom of the wells. If available, use a centrifuge with a plate adapter to briefly centrifuge the plate.

Load and run the reactions

1. Load the reactions into the instrument with the appropriate 7500 Fast Precision Plate Holder on the block:
   - For reactions prepared using plates, place the plate on the sample block.
   - For reactions prepared using 8-tube strips, load the strips vertically in the center of the block.
     If columns 1 and 12 are not used, fill them with empty strips. This will balance the block to avoid damage of tubes.



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

General process for viewing results

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™ dye (target-specific) signal and a VIC® dye (IPC) signal.

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

FAM™ dye signal (target)	VIC® dye signal (IPC)	Result
+	+, –	Positive
–	+	Negative
–	–	See Chapter 4, "Troubleshooting"

3 TaqMan® *E. coli* O157:H7 Detection Kit using the StepOne™ or StepOnePlus™ Real-Time PCR System

Create a run file

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

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

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

For...	Select...
Instrument	**StepOne™ Instrument – (48 Wells)** *or* **StepOnePlus™ Instrument – (96 Wells)**
Type of experiment	**Quantitation – Standard Curve**
Type of reagents	**TaqMan® Reagents**
Ramp speed	**Standard**

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

Tab	Create or select...
Define Targets and Samples	• Create 2 targets with quenchers set to **None**. • Select FAM™ and VIC® dyes as the reporters.[1]
Assign Targets and Samples	Associate the FAM™ and VIC® dyes with each reaction.

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

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

Setting	Stage 1 Enzyme activation	Stage 2 PCR	
Number of cycles	1 (Hold)	40 cycles	
		Denature	Anneal/extend
Temperature	95°C	95°C	60°C
Time	10 min	15 sec	1 min

5. Set Reaction Volume to **30 µL**.

6. Under File, save the run file as a *.eds* document in the appropriate folder.

Set up the PCR reaction

1. Thaw all reagents completely.

2. Prepare the Target Premix Solution: combine the following components for the number of reactions (positive and negative controls) plus 10% overage, and pipet up and down gently to mix.

Component	Vol. for one 30-µL reaction
2X Environmental Master Mix	15.0 µL
10X Target Assay Mix	3.0 µL
Total volume	**18.0 µL**

3. Transfer 18 µL of Target Premix Solution into each well to be used, gently pipetting at the bottom of the well.

4. Add 12 µL of unknown samples and negative controls to each well as appropriate, and gently pipet up and down to mix the solution.

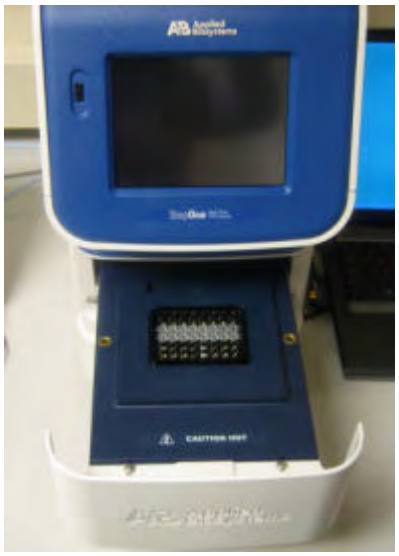
5. Close the tubes or apply an optical cover to the plate. See "How to seal plates for the PCR run" on page 23 or "How to seal tubes for the PCR run" on page 24.

6. Make sure that the reagents are in the bottom of the wells. If available, use a centrifuge with a plate adapter to briefly centrifuge the plate.

Load and run the instrument

For the StepOne™ Real-Time PCR System

1. Load reactions into the instrument:
   - For reactions prepared using the MicroAmp® Fast 48-Well Tray, place tray on the sample block.
   - For reactions prepared using 8-tube strips, load the strips horizontally.
     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.

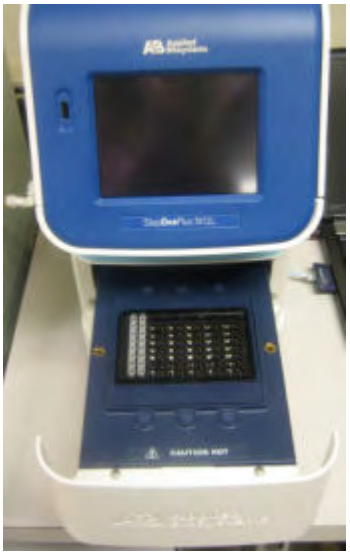


2. Open the run file document that corresponds to the reaction plate that you created in "Create a run file" on page 14.
3. Start the run.

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

For the StepOnePlus™ Real-Time PCR System

1. Load reactions into the instrument:
   - For reactions prepared using the MicroAmp® 96-Well Tray for VeriFlex™ Blocks, place the tray on the sample block.
   - For reactions prepared using 8-tube strips, load the strips vertically.
     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.



2. Open the run file document that corresponds to the reaction plate that you created in "Create a run file" on page 14.

3. Start the run.

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

General process for viewing results

The procedure to view results varies depending on the instrument used. Refer to the instrument user guide for instructions on how to analyze data and view your results.

1. View the amplification plots for all the samples.

2. Set the baseline and threshold values.

3. Check each sample for a FAM™ dye (target-specific) signal and a VIC® dye (IPC) signal.

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

FAM™ dye signal (target)	VIC® dye signal (IPC)	Result
+	+, –	Positive
–	+	Negative
–	–	See Chapter 4, "Troubleshooting"

4 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.
	• Environmental Master Mix not stored properly. • 10X Target Assay Mix not stored properly.	Repeat the assay using properly stored assay components. Avoid freezing and thawing assay components. 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 positive control wells, no IPC or target signal detected	• Environmental Master Mix not stored properly. • 10X Target Assay Mix not stored properly.	Repeat the assay using properly stored assay components. Avoid freezing and thawing assay components. Protect the Target Assay Mix from light.
In positive control 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 positive control wells, no target-specific signal is detected.	Positive control was omitted (pipetting error).	Repeat the assay. Make sure to pipet the positive control into all positive-control wells.

Observation	Possible cause	Recommended action
In negative control wells, no IPC signal is detected, but a target-specific signal is detected.	Carryover contamination caused target signal in negative control wells. Additionally, no IPC signal in negative control wells can be caused by: • A high copy number of target DNA exists in samples, resulting in preferential amplification of the target-specific DNA. • A problem occurred with IPC amplification.	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 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.	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. 2. If the negative control continues to show contamination, repeat the assay using a new kit. 3. If the negative control continues to show contamination, contact Technical Support.
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.

A Supplemental information

Sensitivity

The sensitivity of the PCR is 1–10 copies of the target DNA per reaction. TaqMan® Pathogen Detection Kits are designed to work on enriched samples and detect down to 1 CFU in 25 grams of food.

Specificity

Inclusivity

The TaqMan® *E. coli* O157:H7 Detection Kit has demonstrated 100% inclusivity for 16 strains of serotypes O157:H7, L157:NM, and O55:H7.

Organism	Strain/Source
Escherichia coli O157:H7	ATCC 43890
Escherichia coli O157:H7	380-95
Escherichia coli O157:H7	C7927
Escherichia coli O157:H7	C9490
Escherichia coli O157:H7	96A5997
Escherichia coli O157:H7	96A12165
Escherichia coli O157:H7	96A12374
Escherichia coli O157:H7	96A12814
Escherichia coli O157:H7	43888
Escherichia coli O157:H7	700728
Escherichia coli O157:H7	NTCT 12900
Escherichia coli O157:NM	MF13180-25
Escherichia coli O55:H7	1880
Escherichia coli O55:H7	10591-12-74
Escherichia coli O55:H7	4694-9-75
Escherichia coli O55:H7	2022-7-76

Exclusivity

The TaqMan® *E. coli* O157:H7 Detection Kit has demonstrated 100% exclusivity for 31 *E. coli* strains and 21 non-*E. coli* strains.

Organism	Strain/Source
Escherichia coli	5190
Escherichia coli	4157
Escherichia coli	8739
Escherichia coli	10536
Escherichia coli	11229
Escherichia coli	11775
Escherichia coli	13706
Escherichia coli	13762
Escherichia coli	14948
Escherichia coli	25922
Escherichia coli	33605
Escherichia coli	35218
Escherichia coli	35421
Escherichia coli	51446
Escherichia coli	51755
Escherichia coli	51813
Escherichia coli	9637
Escherichia coli	23848
Escherichia coli	33876
Escherichia coli O26:H11	3359-70
Escherichia coli O145:NM	88.0963
Escherichia coli O78:K80:H12	43896
Escherichia coli O103:H2	87.1368
Escherichia coli O5:NM	85.0587
Escherichia coli O137:H41	88A5333
Escherichia coli O48:H21	94A9057
Escherichia coli O28:H35	96A11759
Escherichia coli O26:H32	ECRC#88-0430
Escherichia coli O55:H9	ECRC#86-0680

Organism	Strain/Source
Escherichia coli O154:H25	94.0726
Escherichia coli O156:H8	93.0541
Bacillus cereus	ATCC 10876
Bacillus cereus	PE1123
Bacillus subtilis	ATCC 11838
Citrobacter freundii	6879
Clostridium perfringens	ATCC 12915
Enterobacter sakazaki	ATCC 51329
Enterococcus faecalis	ATCC 29212
Listeria monocytogenes	7057
Listeria monocytogenes	ATCC 7644
Pseudomonas aeruginosa	ATCC 27853
Pseudomonas aeruginosa	ATCC 17423
Salmonella enteritidis	ATCC 13076
Salmonella enterica	I ser. Infantus
Shigella	Sfla 395
Shigella	SFL 153
Shigella dysenteriae	88.0607
Shigella dysenteriae	ATCC 13313
Staphylococcus aureus	PE491
Streptococcus faecalis	ATCC 9790
Vibrio cholerae	O36
Yersinia enterocolitica	ATCC 9610

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 DNA*Zap*™ 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.

B

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

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Thermo Fisher Scientific, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

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

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- 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

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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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. Department of Agriculture, Food Safety and inspection Service, Microbiology Laboratory Guidebook; go to **http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm**. Accessed 14 Jan. 2014.

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