

USER GUIDE

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TaqMan[®] *Brucella species* Detection Kit

Catalog Number 4382423

Publication Number 4384221

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
C.0	December 2014	<ul style="list-style-type: none">• Updated recommended DNA isolation methods section 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.
B.0	June 2010	Baseline for revision history



Product information

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 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.

Note: We recommend that users perform validation with their unique sample matrices/types to determine appropriate analysis settings (ISO 22174, 2005).

Kit contents and storage

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

Components	Cap color	Volume	Storage ^[1]
10X <i>Brucella species</i> Target Assay Mix ^[2]	Purple	300 µL	Upon receipt, below -18°C. Protect from light. ^[3] Minimize freeze-thaw cycles.
Negative control	White	1000 µL	5±3°C
2X Fast PCR Master Mix	Red	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 (www.lifetechnologies.com). MLS: Fisher Scientific (www.fisherscientific.com) or other major laboratory supplier.

Item	Source
Instrument and equipment	
One of the following real-time PCR systems: <ul style="list-style-type: none"> Applied Biosystems® 7500 Fast Real-Time PCR System Applied Biosystems® StepOnePlus™ Real-Time PCR System Applied Biosystems® StepOne™ Real-Time PCR System 	Contact your sales representative.
Benchtop microcentrifuge	MLS
Pipettors: <ul style="list-style-type: none"> Positive-displacement Air-displacement Multichannel 	MLS
Precision Plate Holder for 7500 Fast System, as appropriate for your experiment	
For plates	Cat. no. 4359652
For 0.1-mL tube strips	Cat. no. 4403809
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
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	

Item	Source
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
MicroAmp® 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

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® *Brucella species* 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 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.

Suggested DNA isolation methods

For most food or environmental sample types, we suggest 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	4428176, 4480466
Spin columns	PrepSEQ® Rapid Spin Sample Preparation Kit with Proteinase K	4426714
Spin columns	PrepSEQ® Rapid Spin Sample Preparation Kit – Extra Clean with Proteinase K	4426715
Spin columns	PrepSEQ® Rapid Spin Sample Preparation Kit	4407760

Workflow

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

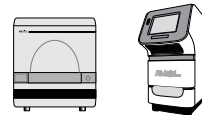


Real-time PCR

7500 Fast Real-Time PCR System (starting page 11)

StepOne™/StepOnePlus™ Real-Time PCR System (starting page 14)

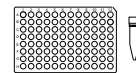
Create a run file



Real-Time PCR instrument



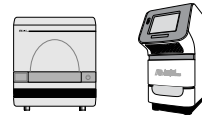
Set up the PCR reactions



96-well plate or tube



Load and run the reactions



Real-Time PCR instrument



View results and analyze data

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.

- 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[®] Brucella species Detection Kit using the 7500 Fast Real-Time PCR System” or Chapter 3, “TaqMan[®] Brucella species Detection Kit using the StepOne[™] or StepOnePlus[™] 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 21 or in “How to seal tubes for the PCR run” on page 22 for sealing the reaction vessels.
- Follow the recommendations in “Good laboratory practices for PCR and RT-PCR” on page 20, including “Plate layout suggestions” on page 20.



TaqMan[®] *Brucella species* 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	
		Denature	Anneal/extend
Number of cycles	1 (Hold)	45 cycles	
Temperature	95°C	95°C	60°C
Time	20 sec	3 sec	30 sec

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

Set up the PCR reactions

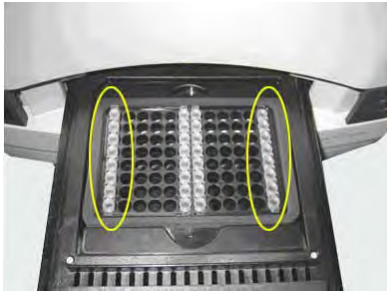
1. Thaw all reagents completely.
2. Prepare sufficient Target Premix Solution according to the following table. Mix by pipetting up and down gently.

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	3 µL	300 µL	27 µL
Total Target Premix Solution	18 µL	1.8 mL	162 µL

3. Transfer 18 µL of Target Premix Solution to the bottom of each well or tube to be used.
4. Add 12 µL of unknown samples and negative controls to each well or tube as appropriate. Gently mix the solution by pipetting up and down.
5. Close the tubes or apply an optical cover to the plate.
6. (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	<p>Load the strips vertically in the center of the block.</p> <p>Note: If columns 1 and 12 are not used, fill them with empty strips. This will balance the block to avoid damage of tubes.</p> 

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™ 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 Appendix A, "Troubleshooting"



TaqMan[®] *Brucella species* 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) <i>or</i> StepOnePlus[™] Instrument – (96 Wells)
Type of experiment	Quantitation – Standard Curve
Type of reagents	TaqMan[®] Reagents
Ramp speed	Fast

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

Tab	Create or select...
Define Targets and Samples	<ul style="list-style-type: none">• 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.

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

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

- Set Reaction Volume to 30 µL.
- Under File, save the run file as a .eds document in the appropriate folder.

Set up the PCR reaction



- Thaw all reagents completely.
- Prepare sufficient Target Premix Solution according to the following table. Mix by pipetting up and down gently.

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	3 µL	150 µL	300 µL	27 µL
Total Target Premix Solution	18 µL	900 µL	1.8 mL	162 µL

- Transfer 18 µL of Target Premix Solution to the bottom of each well or tube to be used.
- Add 12 µL of unknown samples and negative controls to each well or tube as appropriate. Gently mix the solution by pipetting up and down.
- Close the tubes or apply an optical cover to the plate.
- (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.

Instrument	Reaction vessel	Loading method
StepOne™ Real-Time PCR System	48-well plate	Place the plate on the sample block.
	8-well strips	<p>Load the strips horizontally.</p> <p>Note: 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.</p> 
StepOnePlus™ Real-Time PCR System	96-well plate	Place the plate on the sample block.
	8-well strips	<p>Load the strips vertically.</p> <p>Note: 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.</p> 

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™ 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 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.
	<ul style="list-style-type: none"> 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 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: <ul style="list-style-type: none"> 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.

Observation	Possible cause	Recommended action
Replicate results for a sample are inconsistent.	All replicate wells for a sample do not have the same result.	<p>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.</p> <p>If only 2 replicates were run and the results are not consistent, repeat the assay using fresh samples and reagents.</p>



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™ 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/bmb15/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.

Limited product warranty

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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.

For support visit lifetechnologies.com/support or email techsupport@lifetech.com
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