

SureTect™ Brettanomyces spp. and S. cerevisiae Multiplex PCR Assay

USER GUIDE

Direct lysis and real-time PCR detection of *B. naardenensis*, *B. anomalus*, *B. bruxellensis*, and *S. cerevisiae* in swabs, water rinses, and final product samples

for use with:

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v3.0 or later

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Revision D

Revision history: MAN0029758 D (English)

Revision	Date	Description
D	19 March 2026	The Lysis Tubes, Lysis Reagent 3, and Lysis Buffer components were reconfigured to be Lysis Plus Tubes and Lysis Supplement.
C	9 January 2025	- Materials were added for automation, "Materials for automated lysis and qPCR sample preparation for beverage assays" on page 10. - Guidelines were added for automation, "Guidelines for automated sample lysis and qPCR preparation for beverage assays" on page 13. - Instructions were added for automation, Chapter 5, "Prepare the lysate and PCR samples using the automated workflow and perform PCR with the QuantStudio™ 5 Instrument".
B	28 August 2024	Enrichment materials were added for sampling and cultures.
A.0	12 January 2024	New document for the SureTect™ Brettanomyces spp. and S. cerevisiae Multiplex PCR Assay.

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

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Product information

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

Note: These Instructions for Use (IFU) apply to SureTect™ A59289 kits with a component configuration containing Lysis Plus Tubes and Lysis Supplement.

For SureTect™ A59289 kits containing Lysis Tubes, Lysis Reagent 3 Buffer, and Lysis Reagent 3, see the previous version of the IFU.

Product description

Name and intended use

The Thermo Scientific™ SureTect™ *Brettanomyces* spp. and *S. cerevisiae* Multiplex PCR Assay enables real-time PCR detection and differentiation of *Brettanomyces* spp. (*B. naardenensis*, *B. anomalus*, *B. bruxellensis*) and *Saccharomyces cerevisiae* from swabs, water rinses, and final product samples. This assay is intended for use in laboratories undertaking microbiological analysis. See Table 1 for compatible instrument and software.

Note: The qPCR instrument must be calibrated with the following dyes before use: FAM™, ABY™, and Cy5™.

Table 1 Instrument and software

PCR instrument	Software	Pathogen assay file ^[1]
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument	Thermo Scientific™ RapidFinder™ Analysis Software v3.0 or later	<i>Brettanomyces</i> - <i>S. cerevisiae</i> -ST-A59289 assay version 1.0 or later

^[1] Assay files and instructions are available at [thermofisher.com/molecular-microbiology-software](https://www.thermofisher.com/molecular-microbiology-software).

Principle of the test

The assay is based on TaqMan™ real-time PCR technology. Dye-labeled probes target unique DNA sequences specific to *Brettanomyces* spp. (*B. naardenensis*, *B. anomalus*, *B. bruxellensis*) and *Saccharomyces cerevisiae*, and an internal positive control (IPC). Target DNA, if present, is amplified by PCR and detected in real-time using fluorescent hydrolysis probe chemistry. The fluorescence signal that is generated is detected by the real-time PCR instrument and interpreted by the analysis software. For more information about real-time PCR, go to [thermofisher.com/qpcreducation](https://www.thermofisher.com/qpcreducation).

The assay includes an internal positive control for each reaction to confirm that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

Procedure overview

Samples are combined directly with the Lysis Plus Tubes (LPT) and Lysis Supplement (LS), to lyse any yeast cells present in the sample and release their DNA into the lysis solution.

Lysates are transferred to the *Brettanomyces* spp. and *S. cerevisiae* Multiplex PCR Tubes to rehydrate the lyophilized PCR pellet. The PCR tubes are sealed, loaded into the real-time PCR instrument, and the run is started using the RapidFinder™ Analysis software. After the run is complete, the software displays the interpreted results as simple positive or negative symbols. The results can be reported, stored, printed, and downloaded as required.

Results are achieved approximately 60 minutes after loading the prepared sample into the instrument.

Limitations

- The test is designed to detect DNA from target organisms that have been present at a minimum level of 1 CFU/sample and have grown to detectable levels during the enrichment.
- The product is known to be suitable for testing samples such as soft drinks, wine, raw material for beverages, and environmental samples. However, the customer is responsible for the validation of sample matrices, sample preparation, culture media, and enrichment conditions. We recommend testing a selection of known negative and positive samples to ensure that expected results are achieved. See “Test control organisms” on page 27 and EN ISO 22174:2024.
- See Appendix A, “Troubleshooting” for additional information.

Contents and storage

Store the unopened kits at 2–8°C. Store the individual components as indicated in the following table.

Table 2 SureTect™ *Brettanomyces* spp. and *S. cerevisiae* Multiplex PCR Assay Kit, 96 tests (Cat. No. [A59289](#))

Contents	Amount	Storage ^[1,2]
Lysis Plus Tubes (LPT, clear liquid)	12 strips of 8 tubes ^[3] 180 µL each	2–8°C ^[4]
Thermo Scientific™ 96-Well Pierceable Seals	3 sheets	Room temperature (23±5°C)
Lysis Supplement (LS, blue liquid)	1 tube	2–8°C ^[5]
<i>Brettanomyces</i> spp. and <i>S. cerevisiae</i> Multiplex PCR Tubes	12 strips of 8 tubes 1 pellet each	2–8°C Protect from light
PCR Caps	12 strips of 8 caps	Room temperature (23±5°C)

^[1] See the label for the expiration date. Do not use the reagents after the expiration date indicated on the label.

^[2] Opening the kit does not affect shelf life.

^[3] Strips can be cut to select the required number for each run.

^[4] Bring to room temperature before opening.

^[5] –20°C is recommended for long-term storage.

Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or [thermofisher.com](https://www.thermofisher.com). MLS: Fisher Scientific ([fisherscientific.com](https://www.fisherscientific.com)) or other major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

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

Recommended materials for enrichment

Table 3 Equipment, accessories, and consumables

Item	Source
Filterable sample preparation:	
Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with PES Membranes (0.45 µm pore size) or equivalent	124-0045 , 124-0045PK , 165-0045 , 168-0045 or equivalent

Table 3 Equipment, accessories, and consumables (continued)

Item	Source
Homogenizer laboratory blender or diluter, one of the following or equivalent:	
- Homogenizer Laboratory Blender - Diluflux™ Pro Automated Gravimetric Dilutor with simple (non-robotic) dispensing arm - Diluflux™ Pro Automated Gravimetric Dilutor with robotic dispensing arm	DB5000A DB4100A DB4150A
Sample enrichment containers, one of the following or equivalent:	
- BagLight™ 400 (400 mL) - Nunc™ 15 mL & 50 mL Conical Sterile Polypropylene Centrifuge Tubes	DB4013A 339651
Incubators fitted with racks for homogenizer bags	thermofisher.com
Disposable gloves	MLS
Variable volume single-channel pipette, 1- to 10-mL	Available through the Thermo Fisher Microbiology ordering process.
96-well rack	
Filtered pipette tips, 1- to 10-mL	
Sample tubes, 1.5-mL	

Table 4 Additional materials for sampling and cultures

Item	Source
Oxoid™ Sabouraud Liquid Medium EP/USP/JP/BP or equivalent	CM0147B or equivalent
Oxoid™ Sabouraud Liquid Medium EP/USP/JP/BP (Sabouraud Dextrose Broth) (Correx Box) (V6) or equivalent	BO0283M or equivalent
Potato Dextrose Agar or equivalent	BO1010M , BO1010T , or equivalent
Sterile sampling swabs or sponges, for example: Remel™ bio-spo Sponge	- Sponges: R658003 or equivalent - Swabs: MLS

Materials for manual lysis

Table 5 Materials for lysis of enriched cultures

Item	Source
Plastics, consumables, and reagents	
Sample pipette, 1- to 10-mL	Available through the Thermo Fisher Microbiology ordering process.
Adjustable single- or multi-channel pipettor, 5- to 50- μ L and 20- to 200- μ L	
Adjustable single- or multi-channel pipettor, 20- to 200- μ L	
(Optional but recommended) Repeat pipettor, 10- to 100- μ L	
Filtered pipette tips, various sizes	
Thermo Scientific™ 96-Well Pierceable Seals	A55331
MicroAmp™ Adhesive Film Applicator (for pierceable seals)	4333183
Thermo Scientific™ PCR Strip Cutting Tool	A55329
Thermo Scientific™ PCR Capping Tool	A55327
Sample storage tube	A59902 or similar
Additional materials for the thermal cycler method	
Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811
SimpliAmp™ Strip Plate Tray	A55333
Thermo Scientific™ 96-Well PCR Base	A55330

Materials for manual PCR

Table 6 Materials for manual PCR

Item	Source
Real-time PCR instrument and accessories, one of the following instrument packages	
QuantStudio™ 5 Real-Time PCR Instrument, 0.1-mL block, with RapidFinder™ Analysis Software v3.0 or later	A36320 (desktop) A36328 (laptop) Contact your local microbiology sales representative
For the QuantStudio™ 5 Food Safety Real-Time PCR Instrument	
QuantStudio™ 5 Strip Plate Tray	A55332
Thermo Scientific™ QuantStudio™ 5 Tray Cover (optional)	A55972

Table 6 Materials for manual PCR (continued)

Item	Source
Thermo Scientific™ 96-Well PCR Base	A55330
Additional materials for PCR	
Vortex mixer	Available through the Thermo Fisher Microbiology ordering process. See thermofisher.com/plastics for more information.
Plate centrifuge	
8-channel pipette, 10- to 100-µL	
Filtered pipette tips, 10- to 100-µL	
SimpliAmp™ Strip Plate Tray Cover (optional)	A55971
Thermo Scientific™ PCR Strip Cutting Tool	A55329
Thermo Scientific™ PCR Capping Tool	A55327

Materials for automated lysis and qPCR sample preparation for beverage assays

Note: The appliances in Table 7 are used with the SureTect™ Automation Workflow to operate properly.

Table 7 Materials for automated lysis and qPCR sample preparation

Instrument and Appliances	Source
Automation Platform	
Automation Platform Head R 96 ^[1]	A66295
Automation Flex Package	A49008
SureTect™ Automation UV Unit	A49009
SureTect™ Automation Software ^[2]	A66373
Instrument item requirements, not included	
Laptop ^[3]	A49006 or equivalent performing
Automation Starter Pack	A59905
• Automation Lysis Tray	A59019
• Automation PCR Adapter	A59018
• QuantStudio™ 5 Strip Plate Tray	A55332
• Thermo Scientific™ 96-Well PCR Base	A55330
• Thermo Scientific™ PCR Strip Cutting Tool	A55329
• Thermo Scientific™ PCR Capping Tool	A55327

Table 7 Materials for automated lysis and qPCR sample preparation (continued)

Instrument and Appliances	Source
Sample Enrichment Tubes	A59902
Enrichment Tubes Caps	A59903
Filter Tips for SureTect™ Automation 8/96 ^[4]	A59033
Filter Tips for SureTect™ Automation 96 ^[5]	A66298
Waste Bags for SureTect™ Automation	A59034
Additional materials	
Vortex mixer	PT0645 or equivalent
Plate centrifuge	MLS
Nunc™ Adhesive Seals (for cleaning the silicon mat)	236366

^[1] Pipetting head can also be purchased separately (Automation Head R 96 Package, Cat. No. A66297)

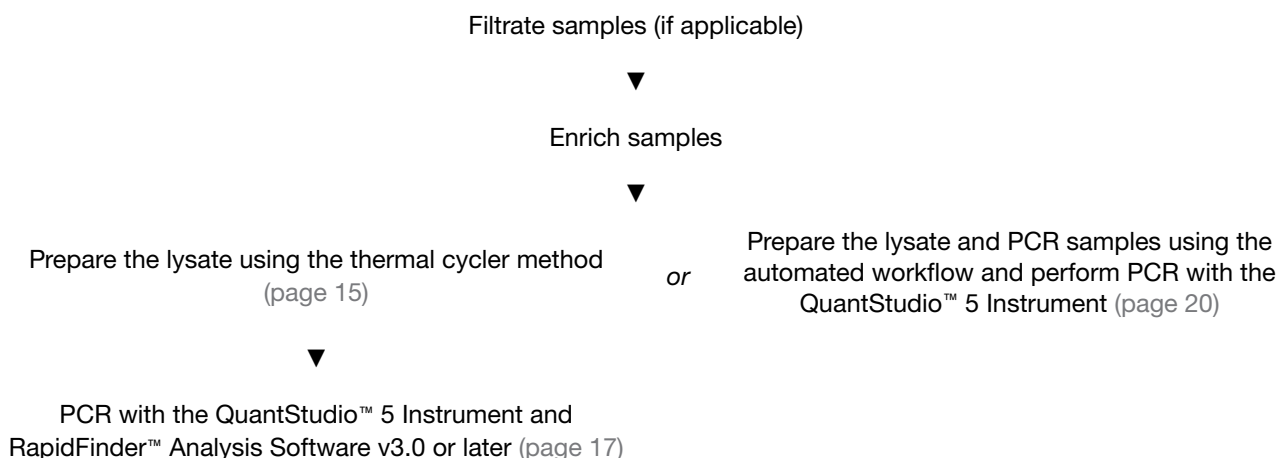
^[2] Available for download at thermofisher.com/molecular-microbiology-software

^[3] Purchase not required. A compatible laptop can be used.

^[4] For use with the Automation Platform Head R 96.

^[5] For use with the Automation Platform Head R 96.

Workflow





Procedural guidelines

Guidelines for sample enrichment

- Follow the manufacturer's instructions for preparation of culture media.
- The recommended enrichment conditions for target detection are 2–5 days at 25°C without shaking. However, the customer is responsible for the validation of sample matrices, sample preparation, and enrichment conditions.
- Comply with Good Laboratory Practices (see EN ISO 7218:2024 standard).
- Dispose of all inoculated culture media as hazardous microbiological waste, even if shown to be negative for the target organism, according to local guidelines.

Guidelines for manual sample lysis

- Ensure that you use the recommended materials.
- Follow the specified temperature and time allowances.
- For downstream PCR on the QuantStudio™ 5 Instrument—Prepare a mock-purified sample using sterile enrichment media as a negative extraction control. (The negative extraction control is optional but highly recommended, to ensure that there is no contamination from raw materials, equipment, or environmental sources).
- Add the enriched sample or negative extraction control to the bottom of the lysis tube.
- For the thermal cycler method, in order to prevent crushing tubes, use the SimpliAmp™ Strip Plate Tray.

Note: When the SimpliAmp™ Strip Plate Tray is used, the detachable side-edges of the 96-well lysis plate must always be broken off and discarded before placing the strips into the tray.

Note: When running a full 96-well lysis plate in the SimpliAmp™ instrument, the SimpliAmp™ Strip Plate Tray is not required.

Guidelines for manual PCR

- Ensure that you use the recommended materials.
- PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
- **IMPORTANT!** After the lysate has been added to the pellets, vortex and spin the pellets and ensure that the pellets rehydrate immediately by tapping the tubes on the lab bench. Start the PCR run within 30 minutes.
- Tube and cap strips can be cut when less than a full strip is required.
Do not cut the strips of caps or tubes too close to the wall of the tube or the cap lid, otherwise the lid might not seal adequately during PCR.
- After the PCR tubes have been opened, add lysate within 10 minutes.
- Ensure that the pellet is fully dissolved. The solution changes from blue to green when the pellet is dissolved.
- For ease of use, a multi-channel pipettor can be used to transfer multiple lysates to the PCR tubes.
- Follow “Good laboratory practices for PCR” on page 29. For more information go to www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.

Guidelines for automated sample lysis and qPCR preparation for beverage assays

Before you begin, thoroughly read the guidelines below in their entirety.

- Ensure that you use the recommended materials.
- Initiate the startup protocols for the QuantStudio™ 5 Food Safety Real-Time PCR Instrument and Automation Platform.
- In all protocols, the plate layouts for the sample tube rack, lysis plate, and PCR plate must be identical.
- For the automated workflow, the enrichments must be done using filter bags. Failure to use filter bags could lead to unsubstantiated results.
- The Sample Enrichment Tubes (Cat. No. A59902 or equivalent performing) must be used to enable correct pipetting height.
- The enriched samples must be placed in the Sample Enrichment Tubes in their specified rack.

Note: The enrichment tubes must contain 400 µL of the enriched sample.

- The CyBio FeliX instrument is not compatible with pierceable seals.
- The Lysis Plus Tubes can be placed into their deck position with or without the Automation Lysis Tray. The Automation Lysis Tray is optional and not mentioned in the deck layout of the SureTect™ Automation Software.
- The caps of the sample tubes and the seals of the Lysis Plus Tubes and Lysis Supplement must be removed before placing them on the deck of the Automation Platform.

- The PCR pellet plate must always be placed into the instrument with the QuantStudio™ 5 Strip Plate Tray and the Automation PCR Adapter for correct pipetting height.
- The seal of the PCR pellet plate must not be removed more than 10 minutes before the workflow is scheduled to start and before placing the plate on the deck of the Automation Platform.
- All accessories listed in the deck layout view of the software must be used.
- Follow “Good laboratory practices for PCR” on page 29. For more information go to www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.

Guidelines for spinning of PCR tubes

- SureTect™ workflow:
 - 20 µL of SureTect™ lysate is added to each PCR tube.
 - The capped PCR tubes are vortexed for 10–15 seconds to ensure that the pellet is fully rehydrated.
 - User must ensure that the reaction mixture is at the bottom of the PCR tube.
- A rapid spin-down is highly recommended before the PCR run to:
 - Collect the reaction mixture at the bottom of the well.
 - Remove bubbles.
- This ensures that the reaction conditions are optimal, and as a result, the PCR step is less likely to fail or to suffer from unwanted signal fluctuations which could affect the interpretation.
- Centrifugation of PCR tubes is included in every GLP (Good Laboratory Practice) protocol.



Prepare the lysate using the thermal cycler method

Before you begin, ensure that you are using the recommended materials (see “Materials for manual lysis” on page 9).

Note: Always wear clean gloves when handling pierceable seals and store pierceable seals in the kit box or in an enclosed bag to prevent contamination of the pierceable seal.

1. Retrieve from storage the Lysis Plus Tubes and Lysis Supplement.
 - a. Place the required number of Lysis Plus Tubes in a Thermo Scientific™ 96-Well PCR Base with the SimpliAmp™ Strip Plate Tray. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.
 - b. Ensure the liquid is collected at the bottom of each tube—flick the tubes in a downward motion or tap the tubes against the bench.
2. Remove the foil from each Lysis Plus Tube and add 10 µL of Lysis Supplement. These tubes are hereafter referred to as prepared Lysis Tubes.

IMPORTANT! Avoid contamination of the Lysis Supplement tube. Always use a new filtered pipette tip each time Lysis Supplement is withdrawn from the stock tube. Failing to do so may result in the loss of functionality of the Lysis Supplement, over time. Use a 10–100 µL repeat pipettor to reduce the number of tips required.

3. Seal the tubes with the Thermo Scientific™ 96-Well Pierceable Seals using the MicroAmp™ Adhesive Film Applicator.

Note: This is an optional step, which is meant to minimize the risk of cross-contamination between samples.

4. Mix and transfer 10 µL of the enriched sample to the prepared Lysis Tube. For the negative extraction controls, transfer 10 µL of sterile enrichment media to a prepared Lysis Tube. Ensure that the pipette tip reaches the bottom of the Lysis Tube to facilitate complete mixing of the sample with the lysis reagents.

IMPORTANT! Mix the enriched sample by vortexing or pipetting; failing to do so may compromise the results of the test.

Note: To prevent cross-contamination, if gloves have been accidentally contaminated with enriched sample, it is highly recommended to change gloves.

5. Seal the tubes with the Thermo Scientific™ 96-Well Pierceable Seals using the MicroAmp™ Adhesive Film Applicator.

IMPORTANT! To prevent crushing the tubes in the SimpliAmp™ Thermal Cycler, use the SimpliAmp™ Strip Plate Tray, or use a full 96-well plate (see "Guidelines for sample lysis").

Note: Whenever the SimpliAmp™ Strip Plate Tray is used, the detachable side-edges of the 96-well lysis plate must always be broken off and discarded before placing the strips into the tray. There is no need to break off the side-edges of the strip plate if a full 96-well plate is used without the tray. When running a full 96-well lysis plate in the SimpliAmp instrument, the SimpliAmp™ Strip Plate Tray is not required.

Note: The SimpliAmp™ Strip Plate Tray Cover should not be used in the lysis step in the SimpliAmp™ Thermal Cycler.

6. Incubate the samples in the SimpliAmp™ Thermal Cycler using the following program settings.

Table 8 Program settings

Step	Temperature	Time
1	37°C	10 minutes
2	95°C	5 minutes
3	10°C	2 minutes
4	4°C	Hold ^[1]

^[1] For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

7. Ensure that the lid heater is on and set to 105°C and the volume is set to Maximum.
8. Carefully remove the Lysis Tubes from the SimpliAmp™ Thermal Cycler. Do not agitate the Lysis Tubes.
9. Proceed directly to Chapter 4, "Perform PCR amplification".

(Optional) Store the samples at 2–8°C for up to 24 hours, including any time stored at 4°C in the thermal cycler.



Perform PCR amplification

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v3.0 or later

Before you begin, ensure that you are using the recommended materials (see “Materials for manual PCR” on page 9).

Set up the plate layout in RapidFinder™ Analysis Software

The plate layout is determined by the user. See the **Help** function in the software for detailed instructions.

In the home screen of the RapidFinder™ Analysis Software, click **Create Experiment**, then enter or edit the well parameters.

Select **Brettanomyces-S. cerevisiae-ST-A59289 assay version 1.0** or later for the assay.

Set up the PCR reactions

Before starting this procedure, see “Guidelines for manual PCR” on page 13.

1. Following the plate layout previously set up in the software, place the required number of *Brettanomyces* spp. and *S. cerevisiae* Multiplex PCR Tubes in the QuantStudio™ 5 Strip Plate Tray. Place the tray on the Thermo Scientific™ 96-Well PCR Base. Ensure the PCR tubes sit firmly in the tray, then tap the tubes on the bench to ensure that the pellets are located at the bottom of the tubes. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.
2. Bring the Lysis Tubes to the bench. A blue layer will be visible at the bottom of the lysate tubes.
Important! Mix the lysates by pipetting up and down three to four times to obtain a homogeneous lysate, failing to do so, may compromise the results of the test.
3. Allow the PCR tubes to remain on the bench for approximately 5 minutes, to bring to room temperature (23±5°C), then open one strip of PCR tubes by removing the seal.

IMPORTANT! If all sample lysates cannot be applied to the PCR tubes in 10 minutes, then open *only one* strip of the PCR tubes, then proceed to the next step.

- If the pellet is collapsed or not pale yellow, do not use.
- If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip. Do not use a tip containing lysate.

4. In the next step, pipette through the seal of the lysis tubes.

Note: The SimpliAmp™ Strip Plate Tray Cover (optional) can be placed over the Lysis Tubes to make pipetting easier.

Note: Make sure to clean the SimpliAmp™ Strip Plate Tray Cover after use to minimize the risk of contamination.

5. Transfer 20 µL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet.

IMPORTANT! Do not touch the pellet when adding the lysate.

6. Seal the PCR tubes with the flat optical PCR Caps provided with the kit.
Ensure that the tubes are properly sealed by pressing down firmly over each opening. Use the PCR capping tool to seal the PCR tubes. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet.
7. If more than one strip of PCR tubes are required, repeat steps 2–5.
8. Mix all PCR tubes thoroughly by vortexing for 10–15 seconds to ensure that the pellet is fully rehydrated.
9. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. It is highly recommended to spin the PCR tubes for at least 10 seconds to remove the bubbles and to collect the liquid at the bottom of the tube before placing in the PCR instrument. Alternatively, hold the tubes upright, and flick sharply downward.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

Load and run the reactions

1. Eject the instrument drawer. Use the QuantStudio™ 5 Strip Plate Tray to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.
2. In the **Run** tab of the experiment file in RapidFinder™ Analysis Software, select the instrument's serial number from the **Instrument** drop-down list.
3. Click **Start Run**, then follow the software prompts.

View results and data analysis

In the home screen of the RapidFinder™ Analysis Software, click **Results**, then click the sub-tab for the desired view of the data.

- **Summary**—plate format
- **Results**—table format
- **Details**—amplification plot

RapidFinder™ Analysis Software results icons

Result icon	Result
	Positive result
	Negative result
	Result warning



Prepare the lysate and PCR samples using the automated workflow and perform PCR with the QuantStudio™ 5 Instrument

Note: These instructions are for automated lysis and qPCR sample preparation using the automated workflow. For manual lysis instructions, see Chapter 3, “Prepare the lysate using the thermal cycler method”.

Note: The instructions in this chapter are for the complete workflow. For all protocols there is the option to set up the run with step-by-step instructions (new users) or with a quick setup (experienced users). The instructions in this chapter are for the quick setup option. For a complete set of detailed instructions for all protocols, see the *SureTect Automation Workflow User Guide*.

Before you begin, ensure that you have read the “Guidelines for automated sample lysis and qPCR preparation for beverage assays” on page 13.

Create the plate layout

The plate layout for the automated workflow must be created in the RapidFinder™ Analysis Software v3.0 or later. See the **Help** function in the software for detailed instructions.

The plate layout file is then exported (in .csv file format) and imported into the SureTect™ Automation Software which controls the Automation Platform.

Note: The Automation Platform uses the same plate layout for the sample tube rack, lysis plate, and PCR plate except for the PCR step only—Selected DNA lysates workflow.

Follow the steps to create the plate layout.

1. Open the RapidFinder™ Analysis Software.
2. Select the assay file.
Assay files and instructions are available at [thermofisher.com/molecular-microbiology-software](https://www.thermofisher.com/molecular-microbiology-software).
3. Type in the Sample ID manually or use a barcode reader.
4. The plate layout must include an assay lot number. If using several boxes of the same lot in one run, they must have unique lot numbers for the instrument to be able to pipette the reagents from the correct tubes, for example, 12345678box1, 12345678box2, etc.

5. Create the plate layout.
6. Export the *.csv plate layout file to a USB drive or the hard drive if the RapidFinder™ Analysis Software and SureTect™ Automation Software are installed on the same computer.

When using the Complete Workflow protocol, plate layouts for the sample tube rack, lysis plate, and PCR plate must be identical.

Prepare the materials for the complete workflow

Before you begin, equilibrate the Lysis Plus Tubes, and *Brettanomyces* spp. and *S. cerevisiae* Multiplex PCR Tubes to room temperature ($23\pm5^{\circ}\text{C}$).

Follow the steps to prepare the materials for the automated procedure.

1. Prepare the Sample Enrichment Tubes
 - a. Transfer 400 μL of the enriched sample to each Sample Enrichment Tube.
 - b. Prepare the Sample Enrichment Tubes in the tube rack according to the plate layout.
2. Prepare the Lysis tray on the laboratory bench.
 - a. Place the Automation Lysis Tray (optional) on the laboratory bench.
 - b. Place the required number of Lysis Plus Tubes on the Automation Lysis Tray (optional) according to the imported plate layout. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.
 - c. Ensure the liquid is collected at the bottom of each tube—flick the tubes in a downward motion or tap the tubes against the bench.
 - d. Allow the tubes to remain at room temperature ($23\pm5^{\circ}\text{C}$) for approximately 10 minutes.
 - e. When prompted by the SureTect™ Automation Software, carefully remove the seals and place the tubes (with or without the lysis tray) in the correct deck position according to the layout.
 - f. When prompted by the SureTect™ Automation Software, carefully remove the cap of the Lysis Supplement and place the reagent in the correct deck position according to the layout.
 - g. When prompted by the SureTect™ Automation Software, carefully place the Sample Enrichment Tubes with the tube rack into the instrument.
3. Prepare the PCR tray on the laboratory bench.

IMPORTANT! Do not open the *Brettanomyces* spp. and *S. cerevisiae* Multiplex PCR Tubes with PCR pellets until instructed to. The tubes must not be opened more than 10 minutes before loading them into the instrument.

- a. Place the QuantStudio™ 5 Strip Plate Tray on top of the Thermo Scientific™ 96-Well PCR Base on the laboratory bench.

- b. Place the *Brettanomyces* spp. and *S. cerevisiae* Multiplex PCR Tubes with PCR pellets on the QuantStudio™ 5 Strip Plate Tray according to the plate layout. Use the Thermo Scientific™ PCR Strip Cutting Tool if less than full strips are needed.
- c. Ensure the PCR tubes sit firmly in the rack, then tap the tubes on the bench to ensure that the PCR pellets are located at the bottom of the tubes.
- d. Allow the PCR tubes to remain on the bench for approximately 5 minutes to bring to room temperature ($23\pm5^{\circ}\text{C}$).
- e. After the lysis step is complete, you will be prompted by the SureTect™ Automation Software that it is time to load the PCR tubes for the PCR sample preparation step. At this point, you can carefully remove the seals.
- f. PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
- g. If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty, pipette tip.
- h. Place the QuantStudio™ 5 Strip Plate Tray with the opened PCR tubes on the Automation PCR Adapter according to the deck layout.

Run the SureTect™ Automation Software

Run the complete workflow - Quick setup

The complete workflow protocol includes the following automated steps:

- Dispensing the lysis reagent (Lysis Supplement) into the Lysis Plus Tubes
 - Dispensing the samples into the Lysis Plus Tubes
 - Lysis incubation step—option to hold at 10°C , then continue with the PCR setup step
 - Dispensing the lysate on the PCR pellet plate
1. Power on the Automation Platform, then run the SureTect™ Automation Software.
 2. At the **Home** screen, select **Complete workflow**.
 3. In the **File import view** screen, select **Quick setup** and import the plate layout file created earlier. The **User ID** is optional.
 4. In the **Lysis Reagents** screen, follow the directions to place the opened Lysis Supplement into the correct position on the adapter.
 5. In the **Deck layout** screen, follow the instructions to place the accessories and consumables in their assigned positions on the decks. Then, select the location of the next available tip in the TipBox on deck positions 2 and 4.
 6. Close the hatch before starting the run.

7. Select **Yes** to start the lysis.

Note: Once the run is started, the screen will show a timer that displays the time remaining until the lysis step is complete.

8. In the next **Deck layout** screen, follow the instructions to place the opened PCR tubes onto the deck.

9. Close the hatch to start the run.

10. Select **Yes** to start the PCR setup.

Note: Once the run is started, the screen will show a timer that displays the time remaining until the PCR step is complete.

11. When complete, remove the PCR tubes, then proceed to seal, vortex, and spin, if needed.

12. Start the PCR run with the QuantStudio™ 5 Real-Time PCR Instrument and RapidFinder™ Analysis Software, using the plate layout created for this sample set. See “PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v3.0 or later” on page 23.

IMPORTANT! Start the PCR run within 30 minutes of addition of sample lysates to the PCR tubes.

PCR with the QuantStudio™ 5 Instrument and RapidFinder™ Analysis Software v3.0 or later

Before you begin, ensure that you are using the recommended materials.

Set up the plate layout in RapidFinder™ Analysis Software

The plate layout is determined by the user. See the **Help** function in the software for detailed instructions.

In the home screen of the RapidFinder™ Analysis Software, click **Create Experiment**, then select the plate layout previously created.

Select **Brettanomyces-S. cerevisiae-ST-A59289 assay version 1.0** or later for the assay.

Load and run the reactions

1. Eject the instrument drawer. Use the QuantStudio™ 5 Strip Plate Tray to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.
2. In the **Run** tab of the experiment file in RapidFinder™ Analysis Software, select the instrument's serial number from the **Instrument** drop-down list.
3. Click **Start Run**, then follow the software prompts.

View results and data analysis

In the home screen of the RapidFinder™ Analysis Software, click **Results**, then click the sub-tab for the desired view of the data.

- **Summary**—plate format
- **Results**—table format
- **Details**—amplification plot

RapidFinder™ Analysis Software results icons

Result icon	Result
	Positive result
	Negative result
	Result warning



Confirm presumptive colonies using the SureTect™ Brettanomyces spp. and S. cerevisiae Multiplex PCR Assay

Confirm colonies using the SureTect™ Brettanomyces spp. and S. cerevisiae Multiplex PCR Assay

The SureTect™ Brettanomyces spp. and S. cerevisiae Multiplex PCR Assay can be used for presumptive colony confirmation.

However, good laboratory practice suggests using different methods to screen and confirm samples. Adhering to this guidance, the SureTect™ Brettanomyces spp. and S. cerevisiae Multiplex PCR Assay should be used either for detection purposes or confirmation of characteristic colonies recovered on agar plates.

When using the SureTect™ Brettanomyces spp. and S. cerevisiae Multiplex PCR Assay to confirm colonies, the sample preparation is as follows:

1. Emulsify an isolated colony in 1 mL of saline.
2. Use this as a test sample by adding 10 µL input and follow the protocol described in Chapter 3, “Prepare the lysate using the thermal cycler method”.
3. Perform PCR as described in Chapter 4, “Perform PCR amplification”.



Troubleshooting

Observation	Possible cause	Recommended action
In negative extraction control wells, target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred.	1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal is detected, but a target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred. Additionally, a problem with the IPC occurred due to: • Preferential amplification of the carryover DNA.	1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment. 2. If the negative extraction control continues to show contamination, repeat the assay using a new kit. 3. If the negative extraction control continues to show contamination, contact Technical Support.
In negative extraction control wells, no IPC signal or an exceptionally weak or atypical IPC amplification plot is detected. The result is considered invalid by the software.	Pellets were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.
	Incomplete lysis steps caused an inhibition of the PCR.	Retest the original sample, ensuring that the correct heating parameters are followed. If the issue persists, contact Technical Support.
In test samples, no IPC nor target-specific signal is detected, and/or an exceptionally weak or atypical amplification plot is observed. The result is considered invalid by the software.	Inhibition of PCR occurred, due to: • PCR inhibitors present in the sample. • Incomplete sample lysis. • Other, unknown, cause.	Repeat the sample lysis procedure and PCR. If the issue persists, contact Technical Support.
	Pellets were not fully dissolved and/or lysate was not at the bottom of the tube before the PCR run was started.	Mix thoroughly by vortexing for 10–15 seconds to ensure the pellet is fully rehydrated and/or lysate is at the bottom of the tube.

Observation	Possible cause	Recommended action
In test samples, no IPC nor target-specific signal is detected, and/or an exceptionally weak or atypical amplification plot is observed. The result is considered invalid by the software. (continued)	Bubbles were present in the PCR tube.	Inspect each tube for bubbles by looking through the optical PCR Caps. Large bubbles can often be removed by firmly holding the top of the tube while gently flicking the bottom. If the bubble persists, spin the tube for 10 seconds in a plate spinner. If the bubble continues to persist, set up a new PCR tube using the prepared lysate.
In test samples, no IPC signal is detected, but target-specific signal is detected. The result is considered invalid by the software.	A problem occurred in IPC amplification due to preferential amplification of the target-specific DNA.	Retest the original sample and diluted sample. Dilute the enriched sample 1:5 (1 part enriched sample and 4 parts sterile media) or 1:10 (1 part enriched sample and 9 parts sterile media), then repeat the sample lysis procedure and PCR.

Test control organisms

Incorporation of positive control organisms is not necessary with routine testing of samples, because the PCR results are validated if the IPC signal is detected. However, you may choose to use target isolates to ensure that the workflow, an assay, and/or a batch performs as it should.

Process a control organism in parallel with test samples through sample enrichment, lysis, and PCR, following your laboratory methodology.

Alternatively, omit the enrichment step for the control organisms: take a single colony into 1 mL of sterile saline to form a homogeneous suspension. Process the prepared suspension through lysis and PCR with test samples.

The following instructions were generated to mitigate the risk of laboratory cross-contamination when handling target strains and provide general guidelines for positive control preparation.

IMPORTANT! Aseptic techniques and sterile consumables should be used at all the times.

- Strain selection and culture:
 - Select a suitable organism.
 - Streak the isolate onto an agar plate to obtain isolated colonies.
 - Incubate the plate under suitable conditions until colonies are visible easily by the naked eye.
- Sample preparation:
 - Pick a single well-isolated colony using a suitable sterile tool (e.g., pipette tip or culture loop).
 - Emulsify the colony carefully in 1 mL of saline or sterile enrichment broth.
 - Dilute the initial suspension using the same medium to obtain C_t value ~ 25-30 (e.g. 1:10 or 1:50).
 - Prepare lysate using 10 μ L of diluted suspension instead of enriched sample. When possible, it is recommended to add the positive sample lysate on the PCR plate only after the (unknown) sample tubes have already been sealed.



- Run PCR according to standard procedure.

Note: Do not open the PCR tubes after the PCR run has completed.



Supplemental information

Good laboratory practices for PCR

Note: Spin tubes/plates before performing PCR. Spinning of PCR tubes is most easily accomplished by using a centrifuge designed for PCR tubes or plates. Follow manufacturer instructions for loading tubes/plates.

To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for 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.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. No. AM9890) according to the Thermo Fisher Scientific PCR Decontamination Protocol. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel. Note that minor discoloration of metal parts may occur.

For additional information, refer to EN ISO 22174:2024 or www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html.

Symbol definitions

Symbol	Definition
	BATCH CODE

(continued)

Symbol	Definition
	CATALOGUE NUMBER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CONSULT INSTRUCTIONS FOR USE
	MANUFACTURER
	TEMPERATURE LIMIT (storage temperature)
	USE-BY DATE



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, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).



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. 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. 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.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also 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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
[cdc.gov/labs/bmbl](https://www.cdc.gov/labs/bmbl)
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
[who.int/publications/i/item/9789240011311](https://www.who.int/publications/i/item/9789240011311)



Documentation and support

Food safety support

Website: <https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html> or [thermofisher.com/foodsafety](https://www.thermofisher.com/foodsafety)

Support email:

- Europe, Middle East, Africa: microbiology.techsupport.uk@thermofisher.com
- North America: microbiology@thermofisher.com

Phone: Visit [thermofisher.com/support](https://www.thermofisher.com/support), select the link for phone support, then select the appropriate country from the dropdown list.

Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation
 - 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.

Related documentation

Document	Publication number
<i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i>	MAN0010407
<i>SimpliAmp™ Thermal Cycler User Guide</i>	MAN0009889
<i>SimpliAmp™ Thermal Cycler Installation and Operation Quick Reference</i>	A24827

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have questions, contact Life Technologies at www.thermofisher.com/support.

References

EN ISO 7218:2024. Microbiology of the food chain – General requirements and guidance for microbiological examinations.

EN ISO 22174:2024. Microbiology of the food chain – Polymerase chain reaction (PCR) for the detection of food-borne pathogens – General requirements and definition.

