

# Calibrate the Cy<sup>®</sup> 5.5 dye for SureTect<sup>™</sup> assay workflows

Use the Applied Biosystems<sup>™</sup> QuantStudio<sup>™</sup> 5 Spectral Calibration Plate (Cy<sup>®</sup> 5.5 dye), 96-well, 0.1mL

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**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

## Overview

This user bulletin describes how to calibrate the QuantStudio<sup>™</sup> 5 Food Safety Real-Time PCR Instrument to use the Cy<sup>®</sup> 5.5 dye. The Cy<sup>®</sup> 5.5 dye is required for use with SureTect<sup>™</sup> assay workflows. To perform a custom dye calibration, use the QuantStudio<sup>™</sup> 5 Spectral Calibration Plate (Cy<sup>®</sup> 5.5 dye), 96-well, 0.1mL (Cat. No. A45218).

## 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.

Table 1

Item	Source
QuantStudio <sup>™</sup> 5 Food Safety Real-Time PCR Instrument, 0.1-mL block, with RapidFinder <sup>™</sup> Analysis Software v1.1 or later	A36320 (desktop) A36328 (laptop) Contact your local microbiology sales representative
Applied Biosystems <sup>™</sup> QuantStudio <sup>™</sup> 5 Spectral Calibration Plate (Cy <sup>®</sup> 5.5 dye), 96-well, 0.1mL	A45218
Vortex mixer	Available through the Thermo Fisher Microbiology ordering process. See <a href="https://www.thermofisher.com/plastics">thermofisher.com/plastics</a> for more information.
Powder-free gloves	
Plate centrifuge	MLS

## Perform a background calibration

Before calibrating for the Cy<sup>®</sup> 5.5 dye, perform a background calibration as outlined in the *QuantStudio<sup>™</sup> 3 and 5 Real-Time PCR Instruments Installation, Use, and Maintenance Guide* or see the [Smart Start E-Learning Portal](#).

## Thaw, vortex, and centrifuge the calibration plate

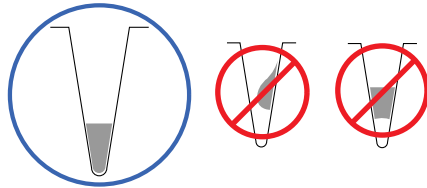
1. Remove the calibration plate from the freezer, then thaw the plate in its packaging. Keep plates protected from light until you perform the calibration.
  - Thaw each plate for 30 minutes.
  - Use each plate within 2 hours of thawing.

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**IMPORTANT!** Do not remove the plate from its packaging until you are ready to use it. The fluorescent dyes in the wells of calibration plates are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.

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2. While wearing powder-free gloves, remove the calibration plate from its packaging and retain the packaging. Do not remove the optical film.
3. Vortex the plate for 5 seconds, then centrifuge at 750–1,000 × *g* for 2 minutes.
4. Confirm that the liquid in each well is at the bottom of the well and free of bubbles. If it is not, centrifuge the plate again.



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**IMPORTANT!** Keep the bottom of the plate clean. Fluids and other contaminants on the bottom of the plate can contaminate the sample block and cause an abnormally high background signal.

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## Add the dye to the software

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**IMPORTANT!** You must add the Cy<sup>®</sup> 5.5 dye to the desktop of cloud software before creating, running, or analyzing experiments that use the Cy<sup>®</sup> 5.5 dye.

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1. In the home screen, touch **Settings ▶ Maintenance and Service ▶ Calibrations ▶ Custom ▶ Custom dye**.
2. To enter information about the new dye, touch **Add Custom Dye**
3. Enter the dye information:
  - Custom Dye Name: **Cy5.5**
  - Type: Select **Reporter**
4. Touch **Save**.

## Perform the dye calibration

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**IMPORTANT!** If you added **Cy5.5 dye** to the dye library in the desktop software or cloud software, you will need to re-enter the **Cy5.5 dye** information in the instrument touchscreen before you perform the **Cy5.5 dye** calibration.

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1. Load the plate.
2. In the home screen, touch **Settings ▶ Maintenance and Service ▶ Calibrations ▶ Custom ▶ Custom dye**.
3. Touch **Cy5.5 dye** to calibrate it.
4. Review the **Cy5.5 dye** information, make changes as necessary, then touch **Update**.
5. Enter the calibration temperature to 60°C.
6. Touch **Start**.

## View calibration results

1. When the run is complete, and the screen displays **Calibration Complete**, touch **View Results ▶ Details**.
2. Review the Calibration plot (see Figure 1) and Calibration QC Status output.  
A passing calibration result, which comes from the highest uniform signal peak in filter X6-m6, will return "The calibration result is fine" output in the Calibration QC Status.

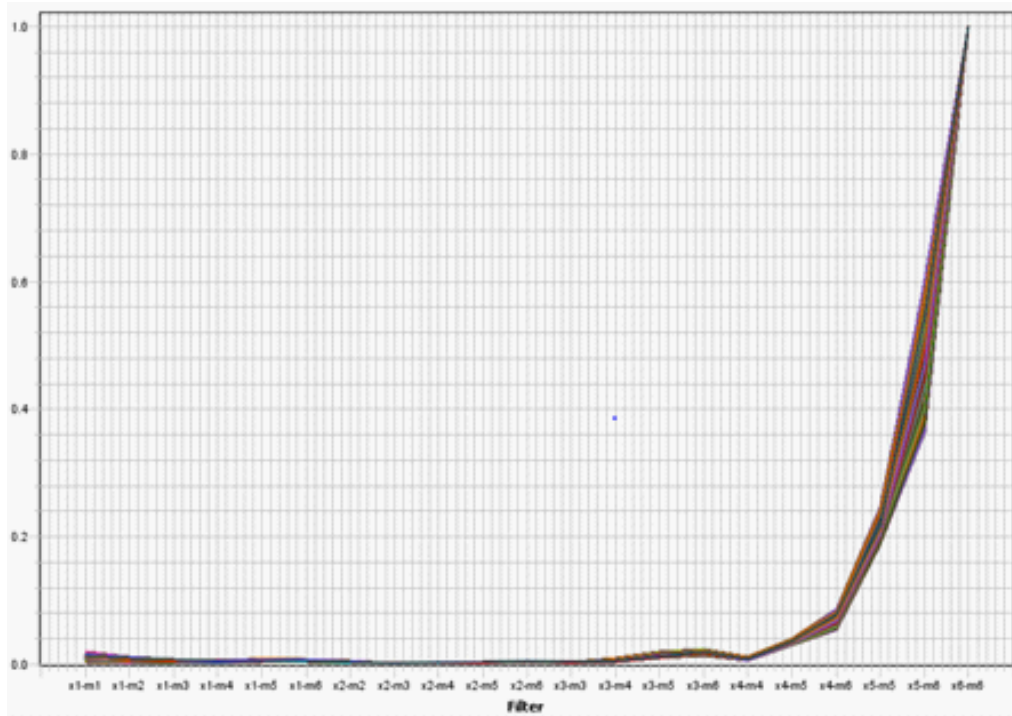


Figure 1 Example Cy<sup>®</sup> 5.5 calibration plot.

3. If the calibration passed, touch **Accept Results**.

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**Note:** Accepting the results saves the calibration data to the instrument and overwrites existing data.

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4. Unload the plate, mark the opening date on the packing sleeve, then store at  $-15^{\circ}\text{C}$  to  $-25^{\circ}\text{C}$ .

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**Note:** Each calibration plate can be used up to 3 times if stored in its packing sleeve at  $-15^{\circ}\text{C}$  to  $-25^{\circ}\text{C}$  and used before the plate's expiration date.

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## Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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

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Revision	Date	Description
B.0	28 April 2021	Updated the View calibration results topic.
A.0	23 January 2020	New document

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