CAL Fluor® Calibration Dyes User Manual

Cat. Nos. 639661 & 639675
PT3859-1 (PR621315)
Published 16 February 2006

Table of Contents

I. Introduction

Spectral calibration is critical for multiplex quantitative PCR (qPCR) assays in order to resolve overlapping fluorescent signals from one another. CAL Fluor Gold Calibration Dye and CAL Fluor Orange Calibration Dye have fluorescent intensities similar to those of other dyes employed in qPCR applications. However, the maxima of the fluorescent emission spectra of CAL Fluor Gold (544 nm) and CAL Fluor Orange (561 nm) permit both dyes to be used in a triplex qPCR assay in combination with FAM (520 nm) in laser-based instruments (e.g., the Applied Biosystems 7900HT Sequence Detection System). Clontech QTox™ Assays and QZyme™ Custom Multiplex Assays employ FAM, CAL Fluor Gold and CAL Fluor Orange dyes as reporters in a triplex assay format on an Applied Biosystems 7900HT instrument.

II. List of Components

Store at –20°C.

The material is sufficient to perform a one-time pure dye calibration on an Applied Biosystems 7900HT or ABI PRISM® 7700 instrument. For either instrument, please consult your instrument's user guide. Most real-time instruments require periodic calibration. For this purpose, after you calibrate your real-time PCR instrument, the reaction plate, containing Cal Fluor Gold and/or Cal Fluor Orange at the proper concentrations, can be frozen at –20°C and reused for later calibrations, for a period of up to one year. Appropriate dilutions must be determined for the Applied Biosystems 7900HT instrument. This Clontech user manual supplements the Applied Biosystems manuals and the dye manufacturer's instructions for the appropriate dye calibration protocols.

CAL Fluor Gold Calibration Dye (Cat. No. 639675)

- 200 µl 5' Cal Gold d(TTT TTT TTT T)3' (6.7 µM)

OR

CAL Fluor Orange Calibration Dye (Cat No. 639661)

- 200 µl 5' Cal Orange d(TTT TTT TTT T)3' (6.7 µM)

III. Additional Materials Required

The following equipment, materials and reagents are required but not supplied.

- Real-time laser PCR instrument (e.g., Applied Biosystems 7900HT)
- TE: 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5
- PCR optical plates (96- or 384-well)
- Optical sealing tape or optical caps

IV. General Considerations

A. Storage and handling

After receipt, the calibration dyes should be stored at –20°C. To ensure maximum performance, calibration dyes should always be protected from light to avoid photobleaching.

B. Calibration and concentration

The concentrations required for calibration vary among different real-time PCR instruments and instrument models. The concentrations we recommend in our protocol were determined through research carried out at Clontech on an Applied Biosystems 7900HT instrument. For other instruments, such as the ABI PRISM 7700 and the Cepheid Smart Cycler II, please refer to the procedures specified by the instrument manufacturer.

C. Centrifuging the plates

Before you put a plate into a real-time PCR instrument, we recommend that you centrifuge the plate briefly (1500 x g for 10 sec) to remove air bubbles and ensure the solution has not collected in the side of a well.

V. CAL Fluor Calibration Dyes Protocol

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

The following is a general protocol for using CAL Fluor Gold and/or CAL Fluor Orange pure dyes for calibration of the Applied Biosystems 7900HT with 384-well plate. For further details, refer to the Applied Biosystems User Guide (Sections 7-5 to 7-30) for the Applied Biosystems 7900HT Sequence Detection System. The protocol consists of three parts: Generating a Background Component, Establishing the Correct Working Concentration for Dye Calibration, and Custom Dyes Calibration.

A. Generating a Background Component

Background can result from background electronic signal, as well as contamination of the sample block, plastic consumables, or water. The Applied Biosystems 7900HT SDS software eliminates the background signal from each sample to maximize the instrument's sensitivity.

1. Dispense 20 µl of deionized water into each well of a 384-well plate. Seal the plate with an adhesive cover.
2. Launch the SDS 2.1 software application. From the **File** menu, select **New.** In assay, select **Background.** In container, select **384 Wells Clear Plate.** In template, select **Blank Template.** Click OK. The software creates a plate document with the attributes for a background run.
3. Select **Instrument Tab,** At the lower portion of **Instrument Tab,** select the **Real-Time Tab.** Click **Connect.**
4. Save the plate document. From **File>Save,** in the **File Name** field of the **Save** dialog box, enter a name such as **Background_051101,** for instance, if the background plate is run on November 1, 2005. Click **Save.**
5. Click **Open/Close** and place the background plate in the instrument tray in the correct orientation.
6. Click **Start** and the instrument will begin the background run.
7. When the instrument finishes the background plate run, the **Run Complete** dialog box appears. Click OK to close the dialog box.
8. Click **Open/Close** and remove the plate from the instrument tray.
9. To analyze the background data, select **Analysis>Extract Background.** If the software displays the **Calibration Update Complete** dialog box, then the run has been successful; if the **Error** dialog box shows, then the run has not been successful, Refer to the Applied Biosystems 7900HT Sequence Detection System User Guide (Section 8-9) for troubleshooting and decontamination information.
10. Save and close the file.

V. CAL Fluor Calibration Dyes Protocol continued

B. Establishing the Correct Working Concentration for Dye Calibration

The procedure in the Applied Biosystems 7900HT Sequence Detection System User Guide requires that you run a serial dilution plate to find an optimized working concentration before undertaking pure dye calibrations.

1. Make a two-fold serial dilution of CAL Fluor Gold or CAL Fluor Orange with TE (pH7.5) to final concentrations of 1,000 nM, 500 nM and 250 nM. Add 20 µl of each concentration into a different well on a 384-well plate. Seal the plate with adhesive film.
2. Start the SDS software, create an allelic discrimination plate document, and run the dilution plate.
3. After the run, select **Analysis > Analyze** and click **Show Raw Data Plot**. In the **Raw Data Plot,** determine the highest concentration of dye that does not produce a saturated signal, and record that concentration for future use. To generate a 45,000 amplitude, which is satisfactory for a pure dye plate run later, we recommend that you use ~450 nM CAL Fluor Gold and 600 nM CAL Fluor Orange, respectively.

C. Custom Dye Calibration

1. Dilute the pure dye with TE (pH 7.5) to a recommended final concentration or the concentration you determined in step B-3. The Applied Biosystems 7900HT instrument requires that each pure dye occupy at least three columns of pure dye plate to permit adequate data collection. For a 384-well plate, 960 µl is needed to fill three columns with 20 µl in each well. We suggest preparing 1,000 µl of pure dye solution for this purpose.
2. Launch the SDS 2.1 software application. From **File** menu, select **New.** In assay, select **Pure Dye.** In container, select **384 Wells Clear Plate.** In template, select **Blank Template.** Then, click OK. The software creates a plate document with the attributes for a pure dye run.
3. Add new dyes using the **Dye Manager**. Select **Tools>Dye Managers,** click **NEW** in the **Add Dye** dialog box and enter a proper name for customer dye, such as CAL Fluor Gold, and click OK. If you want to calibrate CAL Fluor Orange at the same time, repeat the above and click **DONE.**
4. To apply the pure dyes to the customer plate document, select the wells containing the pure dye, select the **Dyes** from the drop-down list in the **Setup Tab,** and select the appropriate dye, such as CAL Fluor Gold.
5. Repeat step C-4 if you would like to calibrate CAL Fluor Orange at the same time.
6. Save the document as .sds format.

V. CAL Fluor Calibration Dyes Protocol continued

7. Select **Instrument Tab** and click **Open/Close.** Place the pure plate in the instrument tray in the correct orientation.
8. Click **Start** and the instrument will begin the pure dye run.
9. When the instrument finishes the pure dye plate run, the **Run Complete** dialog box appears. Click OK to close the dialog box
10. Click **Open/Close** and remove the plate from the instrument tray.
11. To analyze the pure data, select **Analysis>Extract Pure Dye Wizard.** Follow instructions from the **Wizard.** The main purpose is to remove outlying peaks from the pure dye plate document.
12. After inspecting all the wells containing pure dye, save the file and close the program.
13. The instrument is now calibrated for the dye(s) chosen above. You may proceed to use the instrument to collect and deconvolute data with the dye(s).

Note: Appled Biosystems recommends calibrating your instrument every six months, depending on instrument use. We also recommend performing this pure dye calibration every time you recalibrate your instrument with other dyes.

VI. Related Products

For the latest and most complete listing of all Clontech products, please visit **www.clontech.com**

Quantitative PCR Products	Cat. No.
• QZyme Custom Duplex Assays	639669 639670 639671
• QZyme Custom Triplex Assays	639672 639673 639674
• QTox™ ABCB1 ABCC2 GAPDH LSR	638401 638402 638403
• QTox™ ABCC4 ABCC5 GAPDH LSR	638407 638408 638409
• QTox™ ABCC3 ABCC1 GAPDH LSR	638413 638414 638415
• QTox™ NAT1 NAT2 GAPDH LSR	638425 638426 638427
• QTox™ ABCB11 ABCG2 GAPDH LSR	638431 638432 638433
• QTox™ SLCO1B1 SLCO2B1 GAPDH LSR	638437 638438 638439
• QTox™ SLCO1B3 SLC22A7 GAPDH LSR	638443 638444 638445
• QTox™ SLC22A8 SLC22A9 GAPDH LSR	638449 638450 638451

VI. Related Products continued

For the latest and most complete listing of all Clontech products, please visit **www.clontech.com**

Quantitative PCR Products	Cat. No.
• QTox™ SLC22A4 SLC22A5 GAPDH LSR	638455 638456 638457
• QTox™ SLC22A1 SLC22A2 GAPDH LSR	638461 638462 638463
• QTox™ CYP1A2 CYP3A4 GAPDH LSR	638467 638468 638469
• QTox™ CYP2B6 CYP2C9 GAPDH LSR	638473 638474 638475

Additional Related Products

• QZyme MPX FAM	638309 638310
• QZyme MPX JOE	638311 638312
• QTaq™ DNA Polymerase Mix	639651 639652 639655

Notes