PROTOCOL

Pure Dye Calibration for IDT Fluorescent Dyes on the Applied Biosystems 7900 Fast Real-Time PCR System

INTEGRATED DNA TECHNOLOGIES

Please refer to the Applied Biosystems 7900HT Fast Real-Time PCR System Maintenance and Troubleshooting Guide (Part Number 4365542) for instructions and further guidance on pure dye calibration.

Time Required

1 hour

Required Materials

Appropriate dye-labeled calibrating oligonucleotides (available from IDT):
/5Hex/TTTTTTTTTT
/5Joe/TTTTTTTTTT
TE buffer, pH 8.0
96-well optical PCR reaction plates
384-well optical PCR reaction plates
Fast 96-well optical PCR reaction plates
Optical adhesive seal

PART I: Add New Dyes to the Pure Dye Set.

A. Create a Dilution Series Plate for Each New Dye.

1. Prepare a 2X dilution series (Table 1) of the IDT fluorescent dye(s) to be calibrated, using TE buffer, pH 8.0, in a final volume of 2.0 mL.

Calibration Dye	Dilution Series Range (nM)
HEX	25–3200
JOE	25–3200

2. Dispense each of the dilutions into the appropriate reaction plate as follows:
   a. For a **standard 384-well** optical reaction plate, dispense **20 µL** of each of the dilutions in the series into the center wells.

      For a **standard 96-well** reaction plate, dispense **50 µL** of each of the dilutions in the series into the center wells.

      For a **Fast 96-well** reaction plate, dispense **20 µL** of each of the dilutions in the series into the center wells.

   b. Seal the plate using an optical adhesive seal and protect from light until required.

      Note: Fluorescent dyes are photosensitive, and prolonged exposure to light may reduce their fluorescence strength.

B. Create a Plate Document for the Calibration.

1. Open the SDS software application. If required, enter your **User Name** and **Password** and click OK. Select **File > New**. The **New Document** dialog box will open up.
2. Complete the New Document dialog box.
   a. For **Assay**, select **Allelic Discrimination**.
   b. For **Container**, select the appropriate plate format.
   c. For **Template**, select **Blank Template**.
   d. For **Barcode**, leave blank.
   e. Click **OK**. A new plate document will be displayed.
3. Repeat for each dye to be added.

C. Run and Analyze the Dilution Series Plate.

Note: Spin the dilution series plate (from A.2.) at ~1500 x g in a centrifuge with a plate adapter to collect the dye at the bottom of each well.

1. In the plate document, select **Instrument > Plate Read**.
2. Click **Open/Close** to open the instrument tray.
3. Place the dilution series plate into the tray in the correct orientation.
4. Click **Post Read**.
   a. Enter a file name in the **Save As** dialog box and click **Save**.
   b. Click **OK** in response to the message "Document not properly set up. This plate does not contain any marker information. It cannot be analyzed until markers are defined and added to wells."
   c. The plate is loaded and the instrument performs the run.
5. When the run is complete, click **Open/Close** to eject the plate.
6. Click the **Hide/Show System Raw Data Pane** icon  .
7. In the Raw Data Plot, determine the highest concentration of dye that does not produce a saturated signal. Record this concentration. *Saturated signals have high peaks that rise above detectable levels (>65,000 fluorescent units) and appear as plateaus on the plot.*

   The concentration of dye that yields the highest possible signal without saturation is the maximum concentration that can be used with the 7900HT Fast Real-Time PCR System.
8. Repeat steps C.1. through C.7. for each dye.

D. Create a Calibration Plate with the New Dye(s).

1. Prepare 5 mL of each dye at the concentration determined in C.7. (above).
2. Pipet the dye from the previous step (D.1.) into at least 3 columns of a reaction plate.
   - For a **standard 384-well** reaction plate, use **20 µL** per well.
   - For a **standard 96-well** reaction plate, use **50 µL** per well.
   - For a **Fast 96-well** reaction plate, use **20 µL** per well.
3. Seal the reaction plate using an optical adhesive seal and protect the plate from light.

E. Add the New Dye(s) to the SDS Software.

1. Select **Tools > Dye Manager** from the SDS software menu to open the Dye Manager dialog box.
2. In the Dye Manager dialog box, click **New...** to open the Add Dye dialog box.

3. In the Add Dye dialog box:
   a. Enter a name for the new dye and click **OK**.
   b. The new dye appears under Custom in the Dye Manager dialog box.
4. Repeat steps E.1. through E.3. for each new dye included in the calibration plate (at step D.2.).
5. After all of the dyes have been added, click **Done**. The new dye(s) will now be made available to plate documents.

F. Create a Plate Document Template for the New Dye(s).

1. Select **File > New** in the SDS software application to open the New Document dialog box.
2. Complete the New Document dialog box.
   a. For **Assay**, select **Pure Spectra**.
   b. For **Container**, select the appropriate plate format.
   c. For **Template**, select **Blank Template**.
   d. For **Barcode**, leave blank.
   e. Click **OK** to create a new plate document.
3. Apply the new dye(s) to the plate document:
   a. Select the wells containing the first new dye.
   b. Under the **Setup** tab, select the appropriate dye from the dropdown list. This dye will be applied to the selected wells.
   c. Repeat steps F.3.a. and F.3.b. to apply all new dyes to the plate document.
4. Save the document as a plate document template:
   a. Select **File > Save** to open the **Save As** dialog box.
   b. In the **Save in** field, open the **Templates** folder by navigating to **AppliedBiosystems > SDS2.4 > Templates**.
   c. Enter a name for the plate document template in the File Name field.
   d. In the **Files of type** field, select **SDS 7900HT Template Document (*.sdt)** from the dropdown list.
   e. Click **Save** to save the plate document as a plate document template.

PART II: Perform Pure Dye Calibration.

Time required: 30 minutes

G. Create a Plate Document for the New Dye.

1. Open the SDS software application. If required, enter your **User Name** and **Password** and click **OK**. Select **File > New**. The **New Document** dialog box will open up
2. Complete the **New Document** dialog box:
   a. For **Assay**, select Pure Spectra.
   b. For **Container**, select the appropriate plate format.
   c. For **Template**, select the plate document template you created in Part I. F.4. (above).
   d. For **Barcode**, leave blank.
   e. Click **OK** to create a new plate document for pure dye calibration. Do not modify the pure dye plate document.
3. Save the plate document:
   a. Select **File > Save** to open the **Save As** dialog box.
   b. The **Save in** field should show the *SDS Documents* folder. If not, navigate to **Applied Biosystems > SDS Documents**.
   c. In the **File name** field, type in an appropriate file name.
   d. In the **Files of type** field, select **SDS 7900HT Document (*.sds)** from the dropdown list.
   e. Click **Save**.

H. Run A Pure Dye Plate.

1. In the plate document, select the tabs **Instrument > Real-Time**.
2. Click **Open/Close** to open the instrument tray.
3. Place the plate containing the dye to be calibrated into the instrument tray in the correct orientation.

   Click **Start Run** to close the instrument tray and begin the dye calibration. ***Note:*** There may be a slight delay while the heated lid of the instrument is brought to the appropriate temperature.
4. When the **Run Complete** dialog box appears, click **OK** to close the dialog box.
5. Click **Open/Close** to remove the calibration plate from the instrument tray.

   Note: The calibration plate can be stored at –20°C for future use.

I. Analyze the Dye Data.

1. Select **Analysis > Extract Pure Dye Wizard**.
2. Follow the instructions in the Pure Dye Wizard to extract the spectra for your custom dye. For each screen:
   a. Inspect the spectra for shifts in peak location.
   b. Eliminate outlying peaks by selecting the check box of the associated well.
   c. Click **Next**.
   d. Repeat steps a. through c. for all remaining wells. A message prompt will report extraction of the new dyes.

   Data from the dye spectra will be stored as a component of the calibration file.
3. Select **File > Save** to save the plate document for the new dye.
4. Select **File > Close** to close the plate document.
5. Calibration is complete. You can now use the new dye with the instrument.

   Note: As recommended by the instrument manufacturer, calibration should be performed periodically; typically, every 6 months, depending on instrument use.