

# Calibration for Fluorescent Dyes on the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR Systems

## Perform Background Calibration

Perform a background calibration before calibrating your instrument for IDT dyes. Background calibration measures fluorescence intrinsic to the instrument, which will be subtracted from the spectral data of subsequent runs. For 7500/7500 Fast instruments, you must also perform optical calibration. Optical calibration is required to compensate for the effects of the additional filter present in 7500/7500 Fast instruments that is not present in the 7300 instrument. Please refer to the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide (Part Number 4347828 Rev. E) for instructions and further guidance on pure dye calibration.

## Prepare Your Dye(s)

1. Dilute the appropriate dye-labeled calibrating oligonucleotide to the required concentration (see table below) using TE, pH 8.0, buffer in a final volume of 2.0 mL.

<b>Materials:</b>	
Appropriate dye-labeled calibrating oligonucleotide (may be purchased from IDT):	
/5Hex/TTTTTTTTTT	
/5Joe/TTTTTTTTTT	
/5Tet/TTTTTTTTTT	
TE buffer, pH 8.0	
96-well optical PCR reaction plates	

Calibration Dye	Calibration Concentration
HEX	200 nM
JOE	200 nM
TET	200 nM

<b>Estimated time:</b>
7300 - background calibration only: 30 min
7500 - background calibration: 30 min
- optical calibration: 10 min

2. Dispense 20  $\mu$ L of each diluted dye into all wells of an optical PCR reaction plate, using one plate per dye. Seal each plate with an optical adhesive seal and protect from light until required. Fluorescent dyes are photosensitive, and prolonged.

## Create a Plate Document for the Calibration

1. Open the SDS software application. If the Quick Startup dialog box is open, select **Create New Document**. If the Quick Startup dialog box is not open, select **File > New**. The **New Document Wizard** will start up.

Create a new plate document.


- a. For **Assay**, select **Pure Spectra**.
- b. For **Container**, select **96-Well Clear**.  
Exposure to light may reduce their fluorescence strength.
- c. For **Template**, select **Blank Document**.
- d. You can enter your name as **Operator** and include any relevant information in the **Comments** box.
- e. Click the **Finish** button. The **Pure Spectra Calibration Manager** will be displayed.

## Add Custom Dyes to Dye List


1. Click **Add Dye...** to create a new entry for the custom dye.
2. Enter the dye name and click **OK**.
3. Repeat the procedure for each dye to be calibrated.

## Calibrate Your Custom Dyes

1. Select one of the new dyes you have just entered in the Dye List and click **Calibrate**.
  - a. If you are prompted to disconnect the plate document, click **Yes**.
 

 **Note:** Do not click **Yes** or **No** for the message that prompts you to load the calibration plate until you have loaded the reaction plate containing the dye to be calibrated!
2. Check that the dye in each well is at the bottom of the well. If not, centrifuge the plate in a centrifuge with a plate adapter at ~1500 x g.
3. Load the reaction plate containing the dye that you selected under **Dye List** into the instrument according to the manufacturer's instructions, and then click **Yes**. The calibration run will begin and last for ~5 min.
4. When the run is finished, remove the calibration plate from the instrument.
5. To calibrate remaining dyes, click **Next Dye >**.
6. Repeat Steps 3 and 4 for all of the remaining custom dyes you entered in the Dye List.
7. When you have calibrated the instrument with all of your dyes, click **Finish**.

## Analyze the Calibration Data

1. The SDS software creates a plate document for each calibrated dye. From the main Windows menu, select the plate document for one of the calibrated dyes.
  2. Select the **Results** tab, and then the **Spectra** tab.
  3. Select all of the wells by clicking on the upper left corner of the plate map.
  4. Go to the **Analysis** menu, select **Extract Pure Spectra**. The software will complete the extraction and present a plot of the emission spectrum for each of the 96 wells in the plate.
  5. Click **OK** if you get the message **Pure Spectra Extraction Complete**.
  6. In the plate document, select the **Results** tab, and then the **Spectra** tab.
  7. Click on the upper left corner of the plate grid to select all the wells of the plate document.
  8. Check that the peak for the spectrum of the pure dye occurs at the correct filter.
  9. If the peaks for the spectra occur in the correct filter, the calibration is complete and the data is automatically saved as an SDS document file named **Pure Dye** in the directory **Applied Biosystems/SDS Documents**.
-  **Note:** If the peaks for the spectra occur in the wrong filter, check your dyes, and repeat the calibration procedure using the correct dye.
10. Select **File**, and then **Close**. The software displays the plate document for the next dye.
  11. Repeat steps 2 to 10 for each dye that was calibrated.

## Create a Detector for the Pure Dye(s)

1. In the Tools menu of the SDS software, select **Detector Manager**. In the File menu, select **New**. Enter a name and choose a detector color. In the **Quencher Dye** field, select None.
2. Repeat Step 1 for each pure dye, and then click **Done**. Your dye(s) can now be used on this Applied Biosystems real-time PCR instrument.

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