

## Calibration of the ABI 7500 Real-Time PCR System for InnoQuant<sup>®</sup>, InnoQuant<sup>®</sup> H-dye, and InnoQuant<sup>®</sup> HY Fluorescent Dyes using HID Software

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InnoQuant<sup>®</sup> Calibration Kit Product Components:

InnoQuant <sup>®</sup> HEX calibration dye,	1 green top tube	33 µL at 100 µM
InnoQuant <sup>®</sup> Cy3 calibration dye,	1 orange top tube	33 µL at 100 µM
InnoQuant <sup>®</sup> Cy5 calibration dye,	1 red top tube	33 µL at 100 µM
InnoQuant <sup>®</sup> TAMRA calibration dye,	1 brown top tube	33 µL at 100 µM

**Note:** The InnoQuant<sup>®</sup> Calibration Kit is light-sensitive and must be stored in the dark.  
Store the kit at -20°C in a nonfrost-free freezer.

Before using InnoQuant<sup>®</sup>, InnoQuant<sup>®</sup> H-Dye and InnoQuant<sup>®</sup> HY kits, the 7500 instrument must be calibrated for the dyes used in each kit:

<b>InnoQuant<sup>®</sup>:</b>	<b>InnoQuant<sup>®</sup> H-Dye:</b>	<b>InnoQuant<sup>®</sup> HY:</b>
Short Target: FAM	Short Target: FAM	Short Target: HEX
Long Target: Cy5	Long Target: Cy5	Long Target: Cy5
IPC: Cy3	IPC Target: HEX	IPC: TAMRA
		Y Target: FAM

ROX is the reference dye used by the 7500 and should already be calibrated if the instrument is in use.

The dyes are supplied at 100 µM concentration. When preparing a spectral calibration plate for each dye, use TE<sup>-4</sup> as the dilution buffer.

**Note:** It is recommended to use TE<sup>-4</sup> buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA). The pH and EDTA concentration of the TE buffer is critical. TE<sup>-4</sup> may be ordered from Teknova (Cat # T0223).

It is recommended to run the Regions of Interest (ROI) calibration, background calibration, and optical calibration as described in the Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Maintenance Guide before performing the InnoQuant<sup>®</sup> dye calibrations.

## Part 1: Creating calibration dye plates

1. Thaw the 100  $\mu\text{M}$  InnoQuant<sup>®</sup> calibration dyes.
2. Vortex each calibration dye for 10 seconds to mix. DO NOT centrifuge the calibration dyes after mixing.
3. Dilute each calibration dye in a separate tube as described below:

Dye	Final Concentration	Volume of 100 $\mu\text{M}$ dye	Volume of TE <sup>-4</sup> Buffer
HEX	200 nM	10 $\mu\text{L}$	4990 $\mu\text{L}$
Cy3	100 nM	5 $\mu\text{L}$	4995 $\mu\text{L}$
Cy5	50 nM	2.5 $\mu\text{L}$	4997.5 $\mu\text{L}$
TAMRA	50 nM	2.5 $\mu\text{L}$	4997.5 $\mu\text{L}$

4. Vortex each diluted calibration dye for 10 seconds to mix. DO NOT centrifuge the diluted calibration dyes after mixing.
5. Obtain a separate MicroAmp optical 96-well reaction plate for each calibration dye.

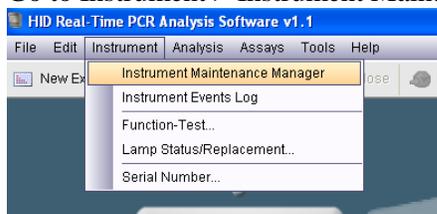
**Note:** Wear gloves at all times when handling the plates and avoid touching the plate wells and adhesive film unnecessarily. Handle the plate by the edges, and avoid touching the bottom or top of the plate.

6. Dispense 50  $\mu\text{L}$  of each dye dilution to all 96 wells of the plate for each calibration dye. Repeat this step for each calibration dye.
7. Seal each plate with MicroAmp optical adhesive film. Protect the plate from exposure to light.
8. Centrifuge plates briefly for 1 minute at 1500 rpm to collect the contents of the wells at the bottom. Proceed to Calibrating a new dye using the HID Real Time PCR Software (Part 2 below).

**Note:** After calibration, store the calibration plates protected from light at  $-20^{\circ}\text{C}$  in a nonfrost-free freezer. Avoid freeze-thawing plates. It is not recommended to use the plates more than 3 times.

## Part 2: Calibrating a new dye using HID Real Time PCR Software

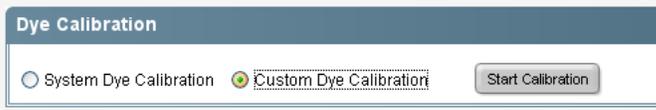
1. Open HID Real-Time PCR Analysis Software.
2. Go to Instrument > Instrument Maintenance Manager



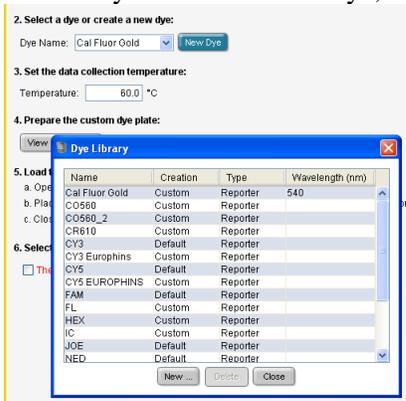
3. Click on the “Dye” tab on the left side of the window.



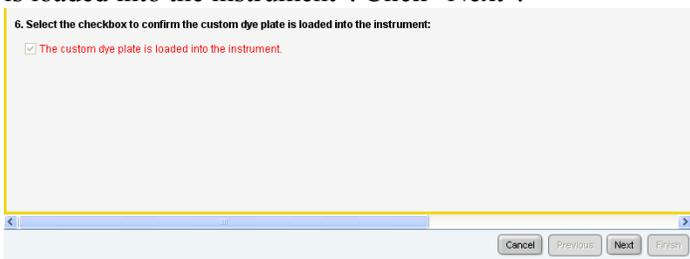
- Click on “Custom Dye Calibration” and then “Start Calibration”.



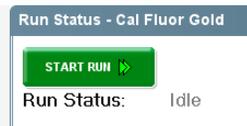
- Click on “New Dye”. When “Dye Library” opens, click “New” then enter dye name. Under Step 2, select a dye or create a new dye, select the dye you have just created.



- Load your custom dye calibration plate into the machine then click the box next to “The custom dye is loaded into the instrument”. Click “Next”.

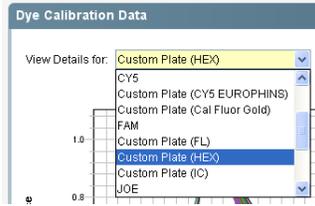


- Click “Start Run”



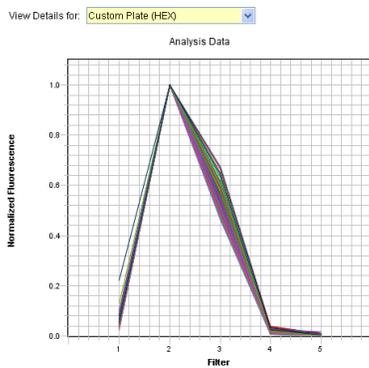
- When run is finished, click “Finish” and repeat Steps 1-7 for each calibration dye to calibrate all the necessary dyes.

- Return to the Instrument Maintenance Manager page and confirm spectra are correct. Go to the dye calibration window pull down window “View Details for:” and select the dye you have just calibrated.

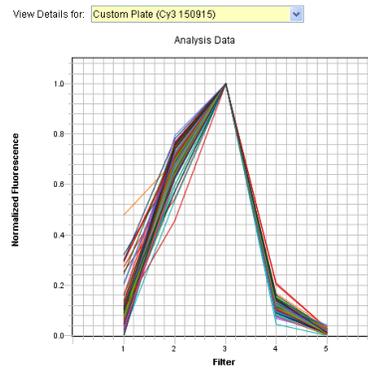


**Note:** HEX should give a signal in Filter 2, Cy3 and TAMRA should give a signal in Filter 3, and Cy5 should give a signal in Filter 5.

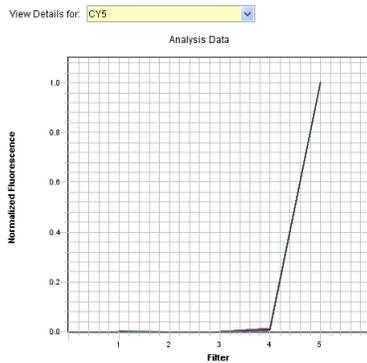
- For HEX, spectra should resemble the following:



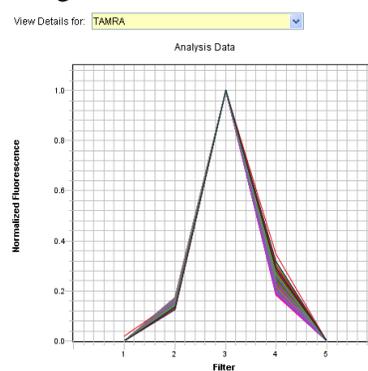
- For Cy3, spectra should resemble the following:



- For Cy5, spectra should resemble the following:



- For TAMRA, spectra should resemble the following:



**Note:** After calibration, store the calibration plates protected from light at -20°C in a nonfrost-free freezer. Avoid freeze-thawing plates. It is not recommended to use the plates more than 3 times.