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CAL FLUOR® AND QUASAR® DYES
Thermal Cycler Spectral Calibration Instructions

WHY IS SPECTRAL CALIBRATION NECESSARY?

In order to successfully use dual-labeled probes containing CAL Fluor® and/or Quasar® dyes in real-time, quantitative PCR (qPCR) multiplexed assays, certain real-time PCR instruments must first be calibrated to recognize the pure dye spectra. Although unnecessary for simple singleplex experiments, spectral calibration is critical for multiplexed assays so that overlapping fluorescent signals can be resolved from one another. This document provides detailed, step-by-step instructions for calibrating these instruments.

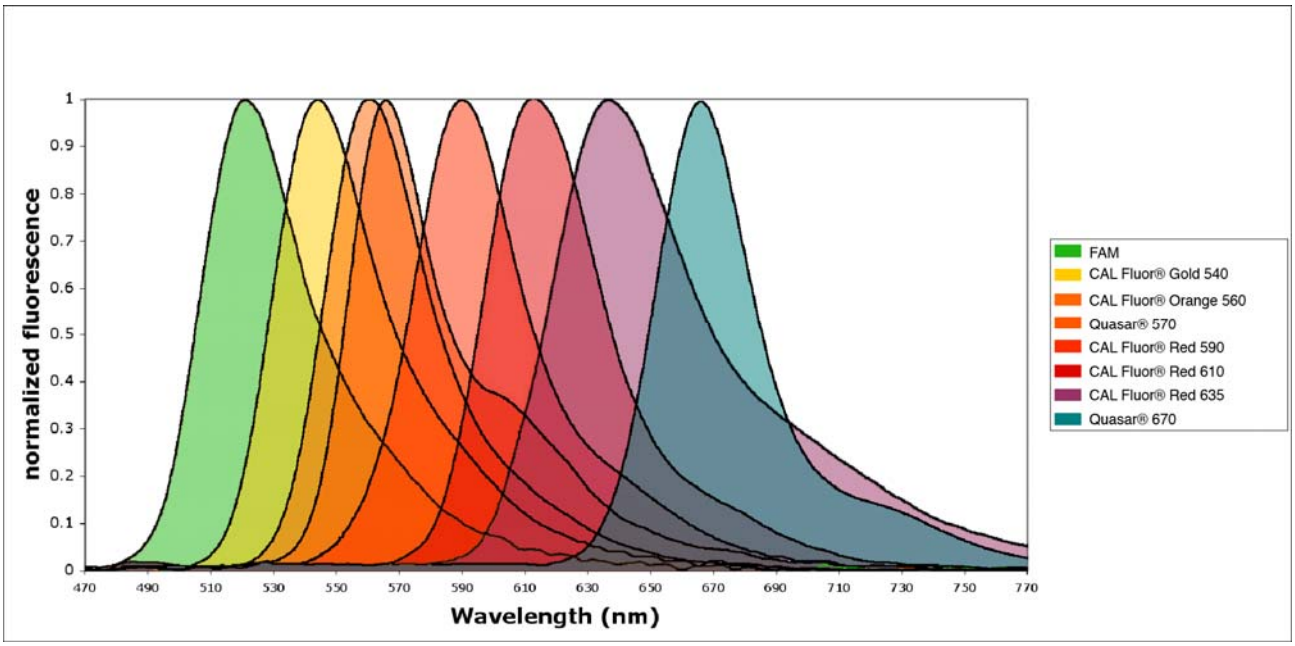
REAL-TIME INSTRUMENTS CURRENTLY SUPPORTED

- ABI **Prism® 7700**
- Bio-Rad **iCycler®**
- Cepheid **SmartCycler® II**
- Bio-Rad **MJ Opticon® 2**

REAL-TIME INSTRUMENTS NOT SUPPORTED

- ABI **Prism 7000**
- ABI **Prism 7300**
- ABI **Prism 7500**
- ABI **Prism 7900**
- Cepheid **SmartCycler**

EMISSION SPECTRA OF CAL FLUOR AND QUASAR DYES



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CALIBRATION DYE—TUBE CONTENTS

Each calibration dye is supplied in an individual tube containing 5 nmol of lyophilized calibration dye covalently linked to a 10 base oligonucleotide comprised of poly T residues (T10).

Biosearch Provides Calibration Dyes in the specifications shown below:

Calibration Dye	Product Number	Amount Provided	US Price
FAM	RD-5025	5 nmol	$95.00
CAL Fluor Gold 540	RD-5080	5 nmol	$95.00
CAL Fluor Orange 560	RD-5081	5 nmol	$95.00
CAL Fluor Red 590	RD-5083	5 nmol	$95.00
CAL Fluor Red 610	RD-5082	5 nmol	$95.00
CAL Fluor Red 635	RD-5084	5 nmol	$95.00
Quasar 570	RD-5063	5 nmol	$95.00
Quasar 670	RD-5065	5 nmol	$95.00

CALIBRATION CONCENTRATIONS

Commercially available real-time thermal cycler models differ substantially in the sensitivity of their optics. Furthermore, several instruments of the same model can also differ in fluorescence detection sensitivity. Calibration concentrations required for each of the CAL Fluor and Quasar dyes will therefore vary according to instrument. Recommended concentrations (determined empirically at Biosearch and with selected scientific collaborators working in the field) are provided within the instrument-specific instructions included below. We suggest that you “fine-tune” these values using the procedure specified by your instrument manufacturer. If your model is not included in this document, please consult the instruction manual that came with your instrument for guidelines on custom dye calibration.

PREPARATION FOR USE

After determining the final working concentration within your instrument-specific instructions, create a stock solution at a higher concentration by re-suspending this calibration dye in your chosen PCR buffer. Store at -20 °C until you are ready to dilute to the working concentration and perform spectral calibration.

STORAGE AND HANDLING

Calibration dyes should be subjected to a minimum number of freeze-thaw cycles. Therefore, we recommend that you prepare microvials, each having sufficient material for one dye calibration and store them frozen at -20 °C or -80 °C. Calibration dyes can be stored frozen in solution for over one year.

PROTECTION FROM PHOTOBLEACHING

To ensure optimum activity and to safeguard maximum performance lifetime, calibration dyes should always be protected from light to avoid photobleaching.

OPTIONAL REACTION PLATE CENTRIFUGATION

When dispensing calibration dyes into a reaction plate, care should be taken to ensure that bubbles are not introduced and that solution hasn’t collected on the side of a well. Briefly centrifuging the plate (<1500xg for 5 seconds) can correct these problems, but otherwise is not required.

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STORING AND SAVING CALIBRATION PLATES FOR FUTURE USE

After successfully calibrating your real-time PCR instrument, the reaction plate containing your calibration dye can be frozen at -20 ˚C and re-used in the future for subsequent calibrations. Most instruments recommend periodic recalibration. Consult your instrument's manual for the recommended frequency of recalibration.

CALIBRATION INFORMATION FOR UNSUPPORTED INSTRUMENTS

For certain real-time PCR instruments we are unable to provide specific calibration instructions or recommended concentrations. If your instrument is one of the following, please refer to the alternate sources of information for the appropriate protocols or contact your instrument manufacturer directly.

ABI Prism 7000 Sequence Detection System

Please refer to the **Sequence Detection System User Guide, Chapter 8 "*Pure Dye Assay (Pure Dyes),*"** pages 8-17 to 8-22.

ABI Prism 7300 Sequence Detection System

Please refer to the document **Installation and Maintenance: Applied Biosystems 7300/7500 Real Time PCR System,** pages 57-72. This document also outlines the procedure for determining the optimum dye concentration for calibration.

ABI Prism 7500 Sequence Detection System

Please refer to the document **Installation and Maintenance: Applied Biosystems 7300/7500 Real Time PCR System**, pages 57-72. This document also outlines the procedure for determining the optimum dye concentration for calibration.

ABI Prism 7900 Sequence Detection System

Please refer to the document **ABI Prism 7900HT Sequence Detection System: User Guide, Chapter 7 Performing a Pure Dye Run,** pages 7-17 to 7-23. This document also outlines the procedure for determining the optimum dye concentration for calibration.

SmartCycler I

Please refer to the **SmartCycler Operator Manual, Appendix B**, and the application note **User-Defined Optical Calibration**. This document also outlines the procedure for determining the optimum dye concentration for calibration.

CUSTOMER SERVICE AND TECHNICAL SUPPORT

If you require additional information or technical assistance, please feel free to email our Technical Support group at:

techsupport@biosearchtech.com

For immediate assistance, our knowledgeable staff is available for telephone consultation from 7:30 AM to 5:00 PM, Monday through Friday, Pacific Coast time. Please contact us at:

1.800.GENOME.1 (436.6631) U.S./Canada only
+1.415.883.8400 telephone
+1.415.883.8488 fax

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INSTRUMENT-SPECIFIC CALIBRATION INSTRUCTIONS

ABI PRISM 7700 SEQUENCE DETECTION SYSTEM

*Following is a general procedure for calibrating Biosearch dyes on the ABI 7700 and a table listing the recommended dye concentrations. For further details, please refer to the **ABI User Bulletin #4: Generating New Spectral Components**, as well as **ABI Prism 7700 User's Manual** that describes the calibration procedure on pages 3-9 to 3-12.*

Calibration Dye	Calibration Concentration on the Prism 7700	Approximate # of Calibrations possible
FAM	200 nM	125
CAL Fluor Gold 540	200 nM	125
CAL Fluor Orange 560	250 nM	100
CAL Fluor Red 590	Incompatible	Incompatible
CAL Fluor Red 610	Incompatible	Incompatible
CAL Fluor Red 635	Incompatible	Incompatible
Quasar 570	Incompatible	Incompatible
Quasar 670	Incompatible	Incompatible

1) Prior to running spectral calibration with your Biosearch dye you will need to generate a background component file. This procedure allows the instrument to measure the background signal of each well and to compensate for any residual fluorescence contaminating the heating block;

2) Dispense 50 µL of deionized water into each well of an optical PCR reaction plate. Seal the plate with an optical adhesive cover and place a compression pad on top of the plate. Position the plate into the instrument and tighten the lid;

3) Start the SDS software application and close the plate layout that opens automatically. Select **New Plate** from the **File** menu, designate the plate type as **Background**, and click **OK**. Click **Show Analysis** and the wells depicted in the plate layout will turn black. Click **Run**;

4) After the run has completed, select **Calibrate** from the **Instrument** menu. Within the **Calibrate** submenu, select **Extract Background Component**. You will then be prompted to quit the application. The background data has been stored regardless of whether you choose to save or discard the file containing the plate setup. You are now ready to prepare your calibration plate containing the Biosearch dye;

5) Dilute the Biosearch Calibration dye(s) in 1X PCR buffer to the necessary working concentration – according to the above table – and a volume of 250 uL. Mix well;

6) For each calibration dye, dispense 50 uL of this dye solution into each of 4 wells in an optical PCR reaction plate. This will leave 50 uL excess which can be discarded. Seal the plate with an optical adhesive cover and place a compression pad on top of the plate. Position the plate into the instrument and tighten the lid;

7) Open the SDS software application, close the existing plate layout and select **New Plate** from the **File** menu. Designate plate type as **Pure Spectra** and click **OK**;

8) Within the **Sample Type** field, select **Sample Type Setup**. You will need to create a new entry for each Biosearch dye by clicking **Add**. A blank entry will be added to the bottom of the list. Within the **Name** field for this entry, type **Pure Dye** exactly as spelled;

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9) Within the **Acronym** field, type a short name for the new dye name using the following conventions: the letter "Z" followed by the acronym that you wish to use. For example, **ZORG** could be used to describe CAL Fluor Orange 560;

   Note: This naming convention is important for the following reasons: the ABI 7700 allows you to calibrate for an unlimited number of custom dyes, but a software bug requires users to maintain FAM, TAMRA, and ROX within the first seven entries alphabetically. This list of calibrated dyes can be viewed by selecting **Edit Pure Dye** within the **Instrument→Calibrate** menus. Adding a Z in front of your dye acronym ensures that this custom entry will be appended to the end of the list and not prevent the software from recognizing the previously mentioned dyes;

10) Make sure that the **Quencher** box is unchecked. Create entries for any additional Biosearch dyes you will calibrate on the same reaction plate, and then exit **Sample Type Setup** by clicking **OK**;

11) In the plate layout, select the wells that correspond to the wells in your reaction plate containing your dye calibration solution. Once these wells are selected, label them as the appropriate Biosearch Pure Dye that is now an entry in the **Sample Type** popup menu. Repeat for any other Biosearch dyes. Click the **Show Analysis** button and your plate layout will turn entirely black. Click **Run** and the instrument will begin collecting the calibration data;

12) When the instrument has completed the data collection, select the four wells in the plate layout that correspond to a calibration dye. Select **Calibrate** from the **Instrument** menu, and then select **Extract Pure Dye**. Click **OK** on the following confirmation screen;

13) You will now be presented with a screen showing the emission spectra of each of your four replicate wells, as well as an emission spectrum representing the average of these replicates. If any of the spectra deviate from the rest of the group, remove these from the average and then click **OK**. Repeat for the other dyes you are calibrating simultaneously. Quit the application;

14) Calibration has been completed and a file titled **Pure Dye** has now been updated to include the new dye(s) in addition to the previous standard dyes. This file is stored in the following directory: **System Folder/Preferences/SDS/Spectra Components**. Importantly, backup this **Pure Dye** file with an appropriate name such as **Master Pure Dye**;

15) The **Pure Dye** file will serve as your 'dye palette' when running future experiments and can be viewed by selecting **Edit Pure Dye** within the **Instrument→Calibrate** menus. Just prior to performing a real-time PCR experiment with the Biosearch dye(s), you will need to delete dye entries that will remain unused during the experiment, using the **Edit Pure Dye** window.

   IMPORTANT NOTE: filtering out unused dyes places the Biosearch dye within the first seven entries, enabling the SDS software to recognize it. This will permanently modify the **Pure Dye** file, which is why the backup described in step 16 is essential. **IF THE PURE DYE FILE HAS NOT BEEN BACKED UP, DELETED ENTRIES CAN ONLY BE REGAINED THROUGH RECALIBRATION!** Due to the software limitation described in step 9, above, the entries for FAM, TAMRA, and ROX should **NEVER** be removed or the application will not open properly.

16) Once you have completed real-time PCR with your Biosearch custom dye, you must restore the dye entries that were removed. Quit the SDS application and replace the current **Pure Dye** file with the **Master Pure Dye** backup file, making the appropriate name changes. Always keep a copy of the **Master Pure Dye** file elsewhere on the computer for future use.

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BIO-RAD LABORATORIES iCYCLER

*Following is a general procedure for calibrating Biosearch dyes on the Bio-Rad iCycler For further details, please refer to the **iCycler iQ™ Real-Time Detection System Resource Guide** that describes the calibration procedure on pages 55-58, and the following table for recommended concentrations.*

Calibration Dye	Calibration Concentration on the iCycler	Approximate # of Calibrations possible
FAM	300 nM	33
CAL Fluor Gold 540	300 nM	33
CAL Fluor Orange 560	300 nM	33
CAL Fluor Red 590	300 nM	33
CAL Fluor Red 610	300 nM	33
CAL Fluor Red 635	300 nM	33
Quasar 570	300 nM	33
Quasar 670	300 nM	33

1) Calibration data for the iCycler is saved to the file **RME.ini** located in the directory **C:\Program Files\Bio-Rad\iCycler\Ini**. By opening this file in a word processor you can view the spectral profile for each of the dyes that are currently calibrated. More information on this file is available in **Appendix I** of the **Resource Guide**. Before performing calibration of any Biosearch dyes, it is important to make a backup copy of this file with an appropriate name such as **Original RME.ini**;

2) To ensure that the iCycler collects spectral data on all relevant channels, It is necessary to anticipate which fluorescent dyes you might use together on a reaction plate and calibrate for all of those simultaneously in one run. For example, if you multiplex a FAM, CAL Fluor Orange 560, and CAL Fluor Red 610 assay, but had calibrated for each of these dyes on separate days, an error message might result stating, “Response Matrix element not found in RME database…” Recalibration is then necessary;

3) Dilute each Biosearch Calibration dye in 1X PCR buffer to the working concentration of 300 nM and a volume of 550 uL. Mix well;

4) For each dye, dispense 50 uL of the Biosearch calibration solution into each of 10 wells in an optical PCR reaction plate. This will leave you with a 50 uL excess for each calibration solution, which you can discard. Seal the plate with an optical adhesive cover and then save it until you are prompted to place it into the iCycler;

5) Start the iCycler software application. In the **Library** mode, check to make sure that you are in the **View Protocol** pane and select the **PureDyeCalibration.tmo** themal cycling protocol;

6) Switch to the **View Plate Setup** pane and select the **Pure dye.pts** plate setup. Click the button **Edit this Plate Setup** and you will be transferred to the **Workshop** mode of the application;

7) Clear the existing well labels by selecting the **Erase** function and clicking the top left corner of the plate layout;

8) Next, select the **Pure Dye** button and label the wells on the plate layout that correspond to the wells in the reaction plate containing the calibration dye(s);

9) Switch to the **Select and load fluorophores** pane and deselect any of the fluorophore entries that are currently checked;

10) The fluorophore entries are labeled with standard dye names followed by the excitation filter through which they are detected. Make a note of those standard dye entries that are coupled with excitation filters appropriate for the Biosearch dye(s) you will be calibrating. Ideally, your choice should be an entry that is infrequently used and whose calibration information can be overwritten to accommodate the Biosearch dye. CAL Fluor Gold 540, CAL Fluor Orange 560, and Quasar 570 are each optimally excited using the 530/30X filter. CAL Fluor Red 590 is optimally excited using the 545/30X filter. CAL Fluor Red 610 is optimally excited using the 575/30X filter. CAL Fluor Red 635 and Quasar 670 are each optimally excited using the 635/30X filter.

    IMPORTANT NOTE: the iCycler does not permit you to create new dye entries beyond their list of standard dyes. Therefore, to use a custom dye on this instrument requires calibrating a standard entry using the custom dye calibration solution. It will be a good idea to leave a note near the instrument outlining the dyes that have been calibrated for the various entries. This calibration will overwrite the previous settings for that entry, which is why the backup described in step 1 is essential! If the need ever arises to use the original dye that was replaced by the Biosearch dye, the backup **RME.ini** file can be substituted for the modified one.

11) Check the box of the standard dye entry for which you will calibrate the Biosearch dye. You will be immediately prompted to select a color representing this dye. On the plate layout, select the wells that you designated as **Pure Dye** and they will be filled in with the color of this entry. Repeat for any additional Biosearch dyes that you will be calibrating simultaneously;

12) After you have designated the location of the calibration dye on your plate layout, click **Save this plate setup** and finally, **Run with selected protocol**;

13) The screen will switch to the **Run Prep** pane, and at this point you should place an **external well factor plate** into the instrument. Preparation of an external well factor plate is described on page 54 of the **Resource Guide**. Select **Begin Run** and the instrument will prompt you to enter a name for the optical data file that will be generated. The iCycler will first collect optical data for the well factors. When the iCycler pauses, open the instrument, remove the well factor plate, and replace it with the reaction plate containing your calibration dyes. Click **Continue Running Protocol**;

14) When calibration has completed, you need to make a backup of the now-modified **RME.ini** file with an appropriate name such as **Current RME.ini**. You are now prepared to run real-time PCR using your custom Biosearch dye on the iCycler. In setting up the plate layouts for these future reactions, remember to use the standard dye entry(s) upon which your Biosearch dyes are calibrated.

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CEPHEID SMARTCYCLER II

Following is a general procedure for calibrating Biosearch dyes on the Cepheid SmartCycler II and a table listing the recommended dye concentrations. For further details, please refer to the ***SmartCycler Application Note: User-Defined Optical Calibration*** *as well as the* ***SmartCycler Operator Manual*** *that describes the calibration procedure in Appendix B.*

Calibration Dye	Calibration Concentration on the SmartCycler II	Approximate # of Calibrations possible (single processing block, 25 µL volumes)
FAM	200 nM	60
CAL Fluor Gold 540	150 nM	80
CAL Fluor Orange 560	100 nM	125
CAL Fluor Red 590	1000 nM	12
CAL Fluor Red 610	200 nM	60
CAL Fluor Red 635	1200 nM	10
Quasar 570	600 nM	20
Quasar 670	300 nM	40

Note: there is considerable variation in the sensitivity of fluorescence detection from one I-CORE® reaction site to the next. While we have strived to determine a universal calibration concentration for each dye, it is possible that these recommendations might produce too low a fluorescent signal to successfully calibrate one or more sites on your instrument. Alternately, the concentration might be too high resulting in a low gain setting and future amplifications that are poorly detected. We recommend fine-tuning these suggested concentrations based on the characteristics of your specific instrument, aiming for a consensus concentration that achieves a gain setting of 2 across most reaction sites.

1) These calibration instructions are specific to 25 µL reaction volumes. If you use different reaction sizes, the recommended concentrations listed in the above table may no longer be appropriate for your application and the instructions will need to be adjusted accordingly;

2) The **SmartCycler Operator Manual** recommends preparing sufficient tubes to simultaneously calibrate all reaction sites on a processing block—16 tubes for each custom dye and an additional 16 tubes containing only buffer (the instruction to prepare 16 tubes that contain a mixture of all dyes is specific to the SmartCycler but unnecessary for the SmartCycler II). With the possibility that our recommended concentrations are unsuitable for your specific processing block, we recommend only preparing enough tubes to calibrate four reaction sites at a time, using these same tubes to sequentially calibrate the remaining sites. Using this strategy to calibrate for a quadraplexed assay would only require 16 tubes in total, and if one dye proves to be inappropriate only four tubes would need to be prepared at a new concentration;

3) All dyes that you plan on using simultaneously as reporters in a multiplexed assay will need to be calibrated in the same run to produce a single “dye set.” If you then change or eliminate one of these reporters in your assay it is strongly recommended that you recalibrate your instrument for this new arrangement;

4) For each custom dye to be calibrated, prepare solutions by diluting into PCR buffer to the appropriate concentration (above table) and a final volume of 125 µL. This should leave you with 25 µL excess. Also prepare 125 µL of PCR buffer alone. Ensure that each of these solutions is uniformly mixed;

5) Dispense 25 µL of each dye solution into each of four reaction tubes. Do the same for the PCR buffer. Label the tubes accordingly and spin them down in the minifuge so that the solution collects into the

diamond reservoir. If any of these tubes have bubbles remaining in the reservoir, spin them down again. Set these tubes aside for future use;

6) Turn on the Smart Cycler II instrument and open the Smart Cycler application. From the **Tools** drop-down menu, select **Optical Calibration**;

7) From the **Dye Set Location** drop-down menu, select one of the two user-accessible entries to calibrate, either **Dye Set-5** or **Dye Set-6**. Type a name for this dye set to remind you which dyes were calibrated and the volumes involved. For example, a 25 µL volume calibration for the dyes FAM, CAL Fluor Orange 560, CAL Fluor Red 610, and Quasar 670 could be titled FORQ25;

8) Select a **Hold Temp** and a **Read Temp** that is identical to the temperature you will be acquiring fluorescence data during your actual real-time PCR amplifications. For a typical TaqMan® 2-step reaction, this is 60 ˚C. Select the 25 µL tube size. The **Hold Time** and the **Read Time** can be left at 10 seconds each;

9) In the **Dyes** pane, type acronyms for the names of the dyes you will be calibrating across the four channels, and the concentration associated with each. FAM should be calibrated on channel 1. CAL Fluor Gold 540, CAL Fluor Orange 560, CAL Fluor Red 590 and Quasar 570 should all be calibrated on channel 2. CAL Fluor Red 610 should be calibrated on channel 3. CAL Fluor Red 635 and Quasar 670 should be calibrated on channel 4. If any channels will be left blank (such as for a duplex or triplex assay) type “unused” or “empty” into the **Name** field of the appropriate channel;

10) In the **Excitation/Emission Mapping** pane, make sure that the wavelength range associated with each channel is correctly positioned for the associated dye. However, if any channel will be left un-calibrated, the excitation and emission mapping for that channel should be left at its default values. Otherwise, channel 1 should have an excitation of 450-495 nm and an emission of 510-527 nm, channel 2 should have an excitation of 500-550 nm and an emission of 565-590 nm, channel 3 should have an excitation of 565-590 nm and an emission of 606-650 nm, and channel 4 should have an excitation of 630-650 nm and an emission of 670-750 nm;

11) Click the **Select Sites** button at the bottom of the window and select the first four reaction sites that you will be calibrating, typically A1-A4. Click **OK**;

12) Click the **Start Calibration** button at the bottom of the window and click **Proceed** at the prompt that follows;

13) A new popup window will appear, prompting you to place the four buffer-only tubes into the appropriate reaction sites. After doing so, click **OK**. Four LED’s associated with the reaction sites you are calibrating should light up;

14) After fluorescence readings of the buffer tubes have been obtained, the instrument will prompt you to replace these tubes with those containing your first calibration dye. After doing so, click **OK**. Follow the instrument prompts for the remaining dyes you will be calibrating. Each time you place and remove tubes from the instrument, use care in handling so that the sides of the tubes are not scratched and bubbles are not introduced into the diamond reservoir. These same tubes will be required later in the procedure;

15) If instrument calibration was successful you will be prompted with the choice to either begin or skip the verification process. This process confirms that the signal intensity of each fluor is as expected, and that bleed-through into adjacent channels can be successfully subtracted out. It is recommended that you conduct verification after calibration. Calibration can fail if either the Gain Normalized Signal (GNS)

or the Signal-to-Background ratio (S/B) is too low. Both of these errors indicate that calibration needs to be repeated with the concentration of one of the dyes boosted to a higher value;

16) The verification procedure will require the same set of tubes to be placed into the instrument again, but it is highly recommended that you shuffle their placement so that the same exact tube will not be placed back into the same exact reaction site. Before beginning verification, we recommend taking this moment to spin them down in the minifuge so that any bubbles introduced during the previous steps can be removed;

17) Follow the prompts during the verification procedure. Upon completion you will be prompted to restart the SmartCycler II application;

18) After the application has finished starting up, we recommend returning to the **Optical Calibration** window and clicking the **Report** button to view the results of the last calibration procedure. Make a note of the **Gains** associated with each channel to understand the intensity of your calibration dyes within the instrument's range of detection. The gains run from 0→3. If you ever witness amplifications that abruptly plateau in your future PCR reactions, this is a sign that the optics have been saturated and that the gain setting was too high for your amplification on that channel. If endpoint fluorescence is important for your data analysis then it will be necessary to recalibrate that channel using a lower concentration that elicits a reduced gain setting;

19) Repeat this calibration procedure (step 6 → step 17) for the remaining 12 reactions sites on the instrument. Once again, use care to not scratch the tubes or introduce bubbles. It is also important to keep all dye names and settings the same as with your previous calibration;

20) Once these are complete you are ready to run your multiplexed assay on the calibrated reaction sites.

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MJ RESEARCH OPTICON 2

*Following is a general procedure for calibrating Biosearch custom dyes on the MJ Research Opticon 2. For further details, please refer to the **DNA Engine Opticon 2 System, Troubleshooting—Calibration,** pages 10-2 through 10-6, that describe the calibration procedure, and the following table recommended concentrations.*

Calibration Dye	Calibration Concentration on the Opticon 2	Approximate # of Calibrations possible
FAM	300 nM	3
CAL Fluor Gold 540	500 nM	2
CAL Fluor Orange 560	1000 nM	1
CAL Fluor Red 590	Incompatible	Incompatible
CAL Fluor Red 610	Incompatible	Incompatible
CAL Fluor Red 635	Incompatible	Incompatible
Quasar 570	Incompatible	Incompatible
Quasar 670	Incompatible	Incompatible

1) The Opticon 2 first measures an empty reaction plate to subtract blank values from the subsequent calibration measurements. Obtain an empty plate of the same type you will use for future real-time PCR reactions and seal it using an optical adhesive cover. Place a compression pad on top of the plate and place it into the instrument;

2) Start the **Opticon Monitor 2** application and select **Dye Calibration** from the **Tools** drop-down menu. You will be presented with a **Dye Calibration Wizard**. In the **Plate type** field, choose the appropriate reaction plate (white or clear). Within the **Dye name** field, type a name for the Biosearch dye that you wish to calibrate, eg. “CALgold.” Click **Next**;

3) Click **Go** and the instrument will begin acquiring the data. Shortly, the instrument will pause and prompt you to rotate the plate 180˚. After doing so, click **Go**;

4) Once the measurements have finished, inspect the results. A problem with the plate will be indicated by anomalous values for one or more wells. If this is observed, discard the plate and start over. Otherwise, click the **Next** button. Note the general signal intensity of the blank plate in each of the detection channels;

5) Remove the empty plate from the instrument and discard the optical cover or caps. Dilute the Biosearch Calibration dye in 1X PCR buffer to the necessary working concentration – refer to **Table 1** - and a volume of 5 mL. Mix well;

6) Dispense 50 uL of this calibration solution into all 96 wells of the same reaction plate that was just removed from the instrument. Seal the plate with a new optical adhesive cover, position a compression pad on top, and place it back into the instrument. Click **Go**. Shortly, the instrument will pause and prompt you to rotate the plate 180˚. After doing so, click **Go**;

7) Once the measurements have finished, again inspect for anomalous results. The signal detected by the dye in at least one of the channels should be over 10X the signal produced by the blank plate in that channel. Importantly, the ratio of intensities of channel 1 to channel 2 should be consistent across the plate. After inspection, click **Next**;

8) Click **Finish** and the calibration procedure is complete. To repeat the procedure for any additional custom dyes that you wish to calibrate on this instrument, click **Yes** at the final prompt.

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PCR Technology

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