iCycler iQ™ Quick Guide
Pure Dye Calibration

The iCycler iQ system is a filter-based optical system that offers sensitive and selective discrimination between multiple fluorophores. An important element of the ability to collect and display data for multiple fluorophores is proper calibration. A short protocol to collect data from pure dye calibrator solutions calibrates the iCycler iQ to separate the overlapping signals of the fluorophores.

Under certain circumstances, it is necessary to recalibrate the system. These include changes in reaction volumes, use of caps vs. optical tape, and addition of new fluorophores for a multiplex reaction. These are changes that affect the optical path and contribution of the pure dye through the filter pairs, and therefore require a new calibration file.

Once a pure dye calibration run is completed, the RME.ini file is automatically updated or rewritten. The RME.ini file is located in C:/Bio-Rad/iCycler/ini. This file contains the calibration data for the software to ensure optimal data for each fluorophore. This RME.ini file can be archived by renaming the file, enabling you to store multiple calibration files. This is useful for laboratories with multiple users who have different experimental needs, such as caps vs. tape.

NOTE: The iCycler iQ software will always use the file named RME.ini that is located at C:/Bio-Rad/iCycler/ini for experimental runs.

What You Will Need

1. iCycler iQ dye calibrator solution kit (catalog #170-8792)

2. 96-well PCR plate (catalog #223-9441) or preferred reaction vessel

3. Optical quality sealing tape (catalog #223-9444) or preferred sealing method

Preparation of a Pure Dye Calibration Plate

1. Pipet equal volumes of the appropriate 1x calibrator solution into 10 wells of the 96-well plate, using the same volumes that you will use for your experiments.

2. You may use up to four pure dye calibrators in separate wells for a single calibration run. For example, a FAM/HEX calibration plate for 50 µl will contain 50 µl FAM calibrator solution in wells B2–B11 and 50 µl HEX calibrator solution in wells C2–C11.

3. Seal the plate with optical tape or strip caps.

Preparation of an External Well Factor Plate

1. Dilute the 10x external well factor solution to 1x with water.

2. In a second 96-well plate, pipet equal volumes of the 1x external well factor solution to the same well locations. For the above example, pipet 50 µl of 1x external well factor solution to wells B2–B11 and C2–C11.

3. Seal the plate in the same manner as the pure dye calibration plate.

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Plate Setup

Create a plate setup file that shows the location of the ten filled wells for each fluorophore being calibrated.

1. In the **Library** module select the **View Plate Setup** tab.

2. Click on **Create a new plate setup**.

3. Select the **Pure Dye** icon in **Edit Plate Setup**.

4. Select the wells that contain the pure dye calibrator solutions.

5. Click on the **Select and load fluorophores** tab. Select the fluorophore to be calibrated. Select a crayon and then select wells that contain that fluorophore. Repeat for each fluorophore.

Sample view   Fluorophore view

6. Click **Save this plate setup**. Name the plate setup and click **Save**.

This plate setup indicates that the FAM pure dye calibration solution is in wells B2–B11 and the HEX pure dye calibration solution is in wells C2–C11.

Protocol Setup

1. In the **Library** module, select the **View Protocol** tab.

2. Select **PureDyeCalibration.tmo** under **Protocol Files**.

Cycle	Repeats	Step	Dwell Time	Hold	Setpoint
1	1	1	01:00		55.0
2	10	1	01:00		55.0

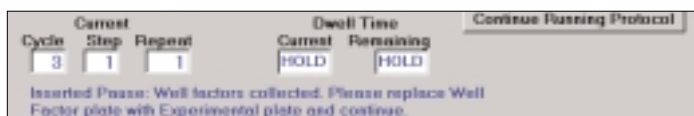Viewing Protocol: PureDyeCalibration.tmo
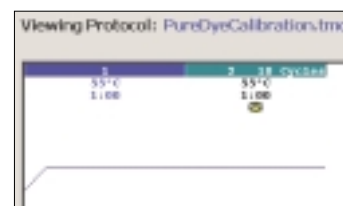
Calibration Run

1. Place the external well factor plate inside the iCycler.

2. Ensure that the thermal protocol and plate setup you created are selected.

3. Click **Run with selected plate setup** from the **View Protocol** tab, or **Run with selected protocol** from the **View Plate Setup** tab. You will be transferred to the **Run Prep** screen.

4. **Pure Dye Calibration** and **Well Factor Plate** will automatically be chosen.

Select run purpose:
- PCR Quantification / Melt Curve
- Pure Dye Calibration — Running plate setup contains Pure Dye sample in one or more wells and only one fluor in each well.

Select well factor source:
- Experimental Plate — Not available during calibration run.
- Well Factor Plate

5. Enter the volume of reagent on the pure dye calibration plate.

6. Click **Begin Run**. Name the optical data file and click **Save**.

7. The iCycler will collect well factors and then alert you to remove the external well factor plate and replace it with the pure dye calibration plate.

Inserted Pause: Well factors collected. Please replace Well Factor plate with Experimental plate and continue.

8. Click **Continue Running Protocol** to execute the pure dye calibration protocol.

9. When completed, the calibration results will be automatically written to the RME.ini file. The calibration may be repeated with additional pure dye calibrators to add additional fluorophores to your RME.ini file.