AMPLIFICATION

Precision Melt Analysis™ Software Quick Guide

Precision Melt Analysis software imports and analyzes data files generated from the CFX96™ or CFX384™ real-time PCR detection system to genotype samples based on the thermal denaturation properties of double-stranded DNA.

Installing Precision Melt Analysis Software

A computer with either the Windows XP or Windows Vista operating system is required to run the software. The software must be installed on the computer by a user with administrative privileges.

To install the Precision Melt Analysis software:

1. Place the Precision Melt Analysis software CD in the computer's CD drive. The software launch page should appear automatically.
2. Click **Next** on the software launch page (Figure 1).
3. Follow the instructions onscreen to complete the installation.
4. If the launch page does not appear automatically, click on **CD drive**, then open and follow the instructions in the Readme.txt file.
5. After installation, launch the software by double clicking on the desktop icon (Figure 2), or select **Start > Programs > Bio-Rad > Precision Melt Analysis**.

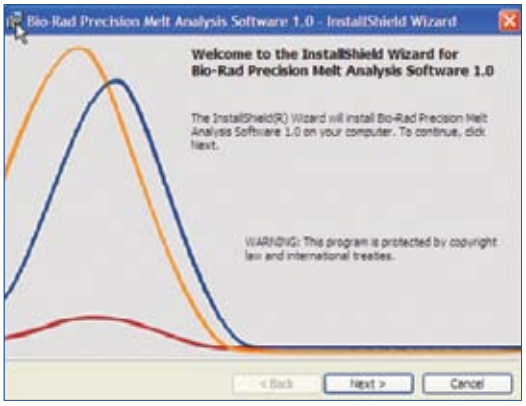


Fig. 1. Welcome screen.

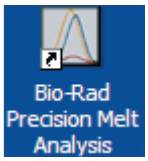


Fig. 2. Desktop software icon.

Melt Calibration

Before Precision Melt Analysis software can analyze data generated on a CFX96 or CFX384 real-time PCR detection system, a melt calibration must be performed. The procedure requires running an experiment and then importing the data into Precision Melt Analysis software to generate a melt calibration file. The software is shipped with a calibration kit that includes the following materials:

- Melt calibration DNA standard
- Melt calibration primers
- SsoFast™ EvaGreen® supermix (catalog #172-5200)

Additional Materials Required

In addition to the components provided in the melt calibration kit, the following materials are required:

- PCR-grade tubes
- Nuclease-free water
- Microseal® 'B' adhesive seals, optically clear (catalog #MSB-1001)
- Hard-Shell® thin-wall 384-well skirted PCR plates with clear shell and white wells (catalog #HSP-3805) for use with a CFX384 system
- Multiplate™ low-profile 96-well unskirted PCR plates with natural wells (catalog #MLL-9601) or white wells (catalog #MLL-9651) for use with a CFX96 system

Preparing a Melt Calibration Plate

1. Add the required volume of each component to an appropriately sized tube (Table 1).
2. Cap the tube and gently mix the reaction components by vortexing.
3. Briefly centrifuge the tube to remove air bubbles and collect contents at the bottom of the tube.
4. Add the appropriate volume of the mixture into each well of a reaction plate:
   - For a CFX96 system, add 20 µl of the reaction mixture to each well of a 96-well plate
   - For a CFX384 system, add 10 µl of the reaction mixture to each well of a 384-well plate
5. Seal the reaction plate with a Microseal 'B' adhesive seal. Centrifuge the plate at 1000 x g for 2 min to move all the reaction components to the bottom of the wells.

Performing a Melt Calibration Experiment

A melt calibration experiment is run on a CFX96 or CFX384 real-time PCR detection system and analyzed using CFX Manager™ software. To run a melt calibration to generate a melt calibration data file:

1. Turn on the CFX96 or CFX384 real-time PCR detection system.
2. Double click the CFX Manager software desktop icon to launch the software.
3. Select **Create a new Experiment** from the list of options in the Startup Wizard (Figure 3). Click **OK** to launch the Experiment Setup window.
4. In the Protocol tab, select **Create New** to open the Protocol Editor.
5. Create the protocol in Table 2.

Table 1. Reaction setup for a melt calibration plate.

Component	Volume for CFX96 System	Volume for CFX384 System
SsoFast EvaGreen supermix	1,200 µl	2,250 µl
Melt calibration DNA standard	120 µl	450 µl
Melt calibration primers	14.4 µl	27 µl
Nuclease-free water	1,065.6 µl	1,773 µl
Total	**2,400 µl**	**4,500 µl**

Fig. 3. Startup Wizard in CFX Manager software.

Table 2. Melt calibration PCR protocol.

Cycling Step	Temperature	Time	Number of Cycles
Enzyme activation	98°C	2 min	1
Denaturation	98°C	5 sec	40
Annealing/extension	55°C	10 sec	
	95°C	1 min	1
	70°C	1 min	1
Melt curve	70–95°C (in 0.2°C increments)	10 sec/step	1

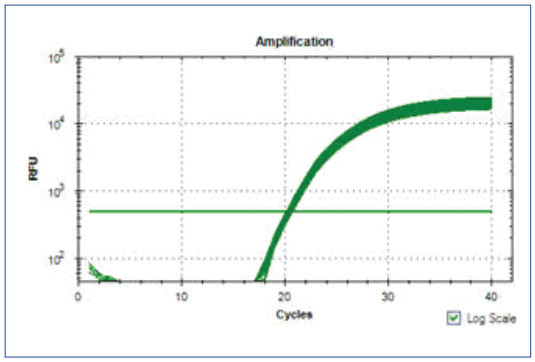
6. Click **OK** to save the protocol and return to the Experiment Setup window.
7. Click the **Plate** tab.
8. Click the **Select Existing > Sample files > MeltCalibration** folder, select the appropriate plate name based on your instrument and plate type, then click **Open**.
   - For a CFX96 system, select **Melt Calibration Plate_ 96 wells_Clear** or **Melt Calibration Plate_96 wells_White**
   - For a CFX384 system, select **Melt Calibration Plate_ 384 wells_White**
9. Click the **Start Run** tab.
10. Select the instrument in the Start Run on Selected Blocks list by clicking the checkbox to the left of the instrument name.
11. Load the melt calibration plate into the instrument.
12. Click **Start Run** to begin running the experiment on the selected block.
13. At the prompt, save the name of the melt calibration data file as Melt Calibration_run date, for example, Melt Calibration_050109.
14. When the melt calibration run is complete, CFX Manager software automatically opens and processes the data file. Check the data file to ensure all wells display a tight amplification and a single melt peak (Figure 4).

Importing a Melt Calibration File

Precision Melt Analysis software is used to open data files that have been generated from an experiment performed on a CFX96 or CFX384 real-time PCR detection system and analyzed using CFX Manager software. To open and generate a melt calibration file using Precision Melt Analysis software:

1. Launch Precision Melt Analysis software by double clicking on the Precision Melt Analysis software icon on the Desktop.
2. Click **Tools > Import Melt Calibration** from the Menu bar (Figure 5).
3. Choose the name of the melt calibration experiment data file (.pcrd extension) and click **Open**.
4. A window will appear indicating the calibration was successful.
5. Click **OK** to proceed and use Precision Melt Analysis software.

A



B

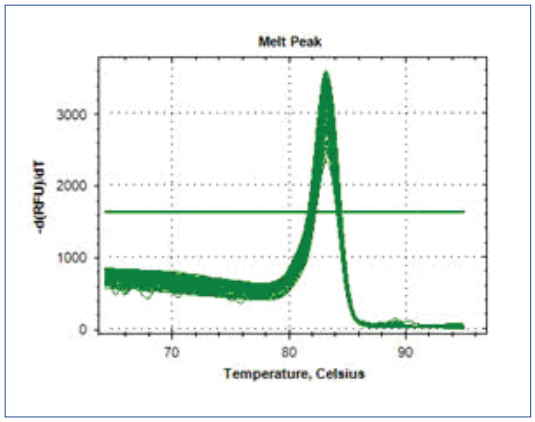


Fig. 4. Melt calibration data. A, amplification plot; **B**, melt curve plot with a single peak.

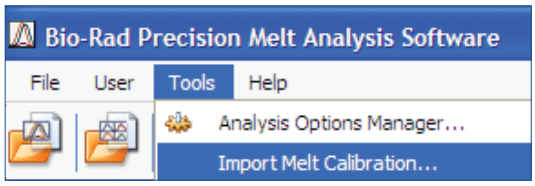


Fig. 5. Import Melt Calibration file.

Software Navigation

Double click on the desktop icon (Figure 2), or select **Start > Programs > Bio-Rad > Precision Melt Analysis**. To navigate within the software, use the Startup Wizard (Figure 6), which provides quick access to common software commands found in the menu bar and the toolbar.

Converting a Data File to a Melt File

To convert a data file to a melt file, select **Create a new melt file** in the Startup Wizard (Figure 6), or select **File > New > Melt File** from the File menu. Choose the data file, then click **Open**.

To open an existing melt file, select **Open a melt file** in the Startup Wizard (Figure 6), or select **File > Open > Melt File** from the File menu.

Analyzing and Viewing Data

Precision Melt Tab

When you open a melt file in the Precision Melt Analysis software, the software uses the default analysis settings to assign a cluster to each sample. To analyze the data in the different panes:

1. In the Melt Curve pane, select the **Normalized view** checkbox to compare all the curves with the same starting and ending fluorescent signal level applied. Two cursors per region are provided, defaulted to the ends of the curve, as shown in Figure 7.
2. **Optional:** In the Melt Curve pane, select **Temperature-shifted view** to apply a temperature shift to each normalized fluorescence curve along the temperature axis (x-axis) (Figure 7).
3. For easy visual identification of clusters, Precision Melt Analysis software generates a difference curve for each well (Figure 8). The Difference Curve chart shows the difference in fluorescence between a well and the fluorescence of a reference curve.
4. Right click on the fluorescence trace of a well in the Melt Curve or Difference Curve chart to change the selected sample or selected cluster parameters (Figure 9).

Tip: Right click on the spreadsheet for options. Export the data in the spreadsheet by right clicking and selecting the **Export to Excel** option.

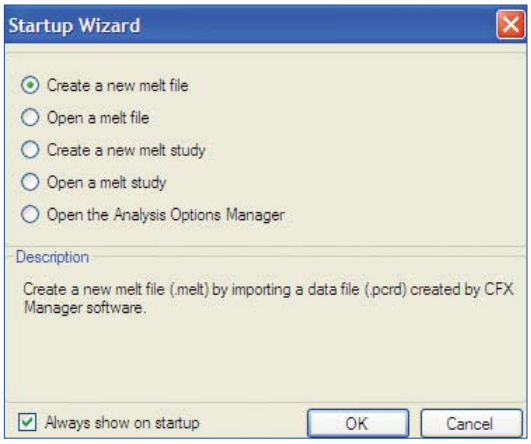


Fig. 6. Startup Wizard in Precision Melt Analysis software.

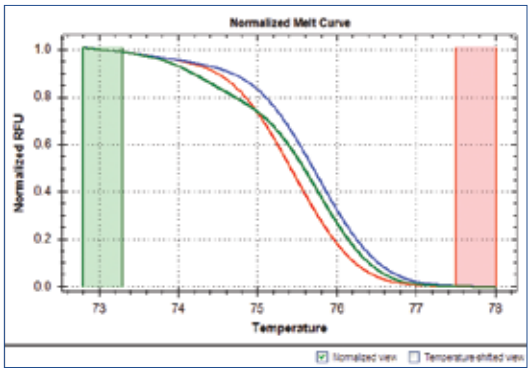


Fig. 7. Melt Curve pane.

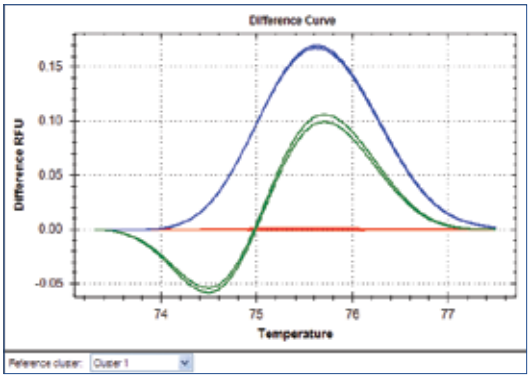


Fig. 8. Difference Curve pane.

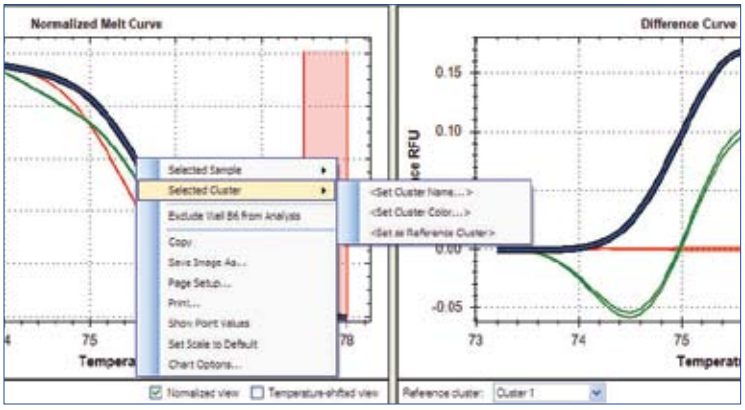


Fig. 9. Changing cluster parameters.

Viewing Well Group Data

Use the Well Group drop-down menu on the toolbar to view data for a specific well group (Figure 10). The software treats well groups as independent experiments, and each well group can be analyzed with its own settings.

Viewing Results

Click the **Precision Melt Data** tab in the Data Analysis window (Figure 11) to view the numerical data calculations and statistics. Click on any column header to sort rows based on that column. Right click on the spreadsheet and select **Sort** to sort rows based on multiple columns. Display data in the following ways:

- Right click on the spreadsheet, and select a format to export data as a Microsoft Excel, image, XML, or HTML file
- Select **Plate View** from the drop-down menu under the Precision Melt Data tab to display well data in a plate grid format
- Select **Charts** from the drop-down menu under the Precision Melt Data tab to display all charts

Creating a Report

To create a report follow these steps:

1. Click **Report** on the toolbar of the Data Analysis window to open the Report window.
2. Click the applicable checkboxes in the report options list to include specific information in the report, which is displayed in the preview section (Figure 12).
3. Click **File** on the toolbar and select **Save** to save the report as a PDF, or select **Print** to print the report.
4. Save the report template for use with other data files, if desired.

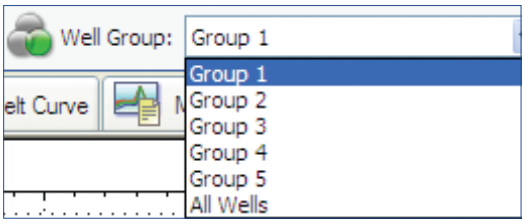


Fig. 10. Well Group selection.

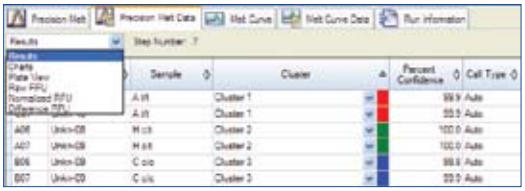


Fig. 11. Precision Melt Data tab.

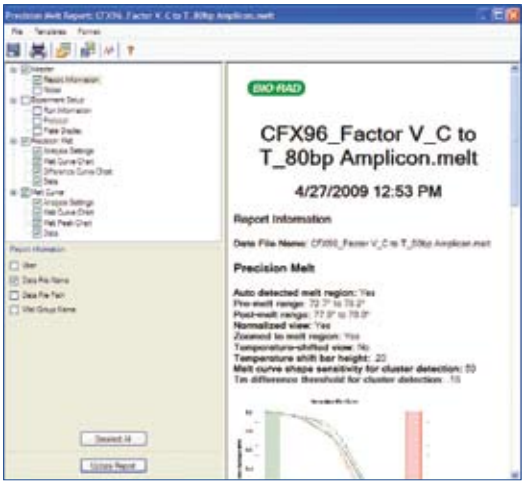


Fig. 12. Report window. Report options list includes checkboxes for choosing information to include in a report.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 364 3435* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

10014810 Rev A US/EG *08-0864 0509 Sig 0308*