

Precision Melt Analysis Software

Quick Guide

Precision Melt Analysis Software imports and analyzes data files generated from the following CFX Real-Time PCR Systems to genotype samples based on the thermal denaturation properties of double-stranded DNA.

- CFX Opus 96
- CFX Opus Deepwell
- CFX Opus 384
- CFX Duet
- CFX96 Touch
- CFX96 Touch Deep Well
- CFX384 Touch
- CFX Connect
- CFX96
- CFX384

Installing Precision Melt Analysis Software

A computer with a Windows 10 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 **CD drive**, then open and follow the instructions in the Readme.txt file.
5. After installation, make sure a valid HASP key is inserted into the USB port of your computer before launching the software. Launch the software by double clicking the desktop icon (Figure 2), or select **Start > Programs > Bio-Rad > Bio-Rad Precision Melt Analysis**.

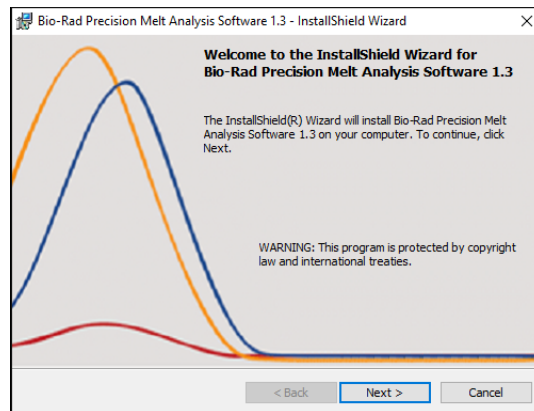


Fig. 1. Welcome screen.



Fig. 2. Desktop software icon.

Software Navigation

To navigate in the software after launch, use the Startup Wizard (Figure 3), which provides quick access to common software commands found in the menu bar and the toolbar.

Melt Calibration

Before Precision Melt Analysis Software can analyze data generated on a CFX Real-Time PCR 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
- Precision Melt Supermix (catalog #1725110)

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' PCR Plate Sealing Film, optically clear (#MSB1001)
- Hard-Shell™ Thin-Wall 96-Well Skirted PCR Plates with white shell and clear wells (#HSP9601) or white wells (#HSP9655) for use with a CFX Opus 96, CFX96 Touch, CFX96, CFX Opus Deepwell, CFX96 Touch Deep Well, CFX Duet, or CFX Connect System
- Hard-Shell Thin-Wall 384-Well Skirted PCR Plates with clear shell and white wells (#HSP3805) for use with a CFX Opus 384, CFX384 Touch, or CFX384 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 CFX Opus 96, CFX96 Touch, CFX96, CFX Opus Deepwell, CFX96 Touch Deep Well, CFX Duet, or CFX Connect System, add 20 µl of the reaction mixture to each well of a 96-well plate
 - For a CFX Opus 384, CFX384 Touch, or 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' PCR Plate Sealing Film. Centrifuge the plate at 1,000 x g for 2 min to move all the reaction components to the bottom of the wells.

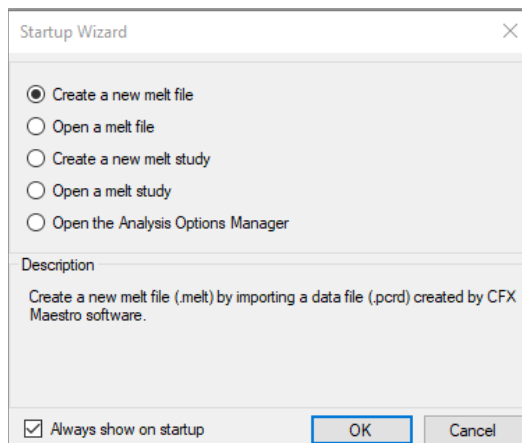


Fig. 3. Startup Wizard in Precision Melt Analysis Software.

Table 1. Reaction setup for a melt calibration plate.

Component	Volume for 96-Well CFX System, µl	Volume for 384-Well CFX System, µl
Precision Melt Supermix	1,200	2,250
Melt calibration DNA standard	120	450
Melt calibration primers	14.4	27
Nuclease-free water	1,065.6	1,773
Total	2,400	4,500

Performing a Melt Calibration Experiment

A melt calibration experiment is run on a CFX Real-Time PCR System and analyzed using CFX Maestro Software. To run a melt calibration to generate a melt calibration data file:

1. Turn on the CFX System.
2. Double click the CFX Maestro Software desktop icon to launch the software.
3. Select **User-defined** from the Run setup tab of the Startup Wizard (Figure 4) to launch the Run Setup window.
4. In the Protocol tab, select **Create New** to open the Protocol Editor.
5. Create the protocol in Table 2.
6. Click **OK** to save the protocol and return to the Run 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 96-well system, select **Melt Calibration Plate_96 wells_Clear** or **Melt Calibration Plate_96 wells_White**
 - For a 384-well 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_121522.
14. When the melt calibration run is complete, CFX Maestro Software automatically opens and processes the data file. Check the data file to ensure all wells display a tight amplification curve and a single melt peak (Figure 5).

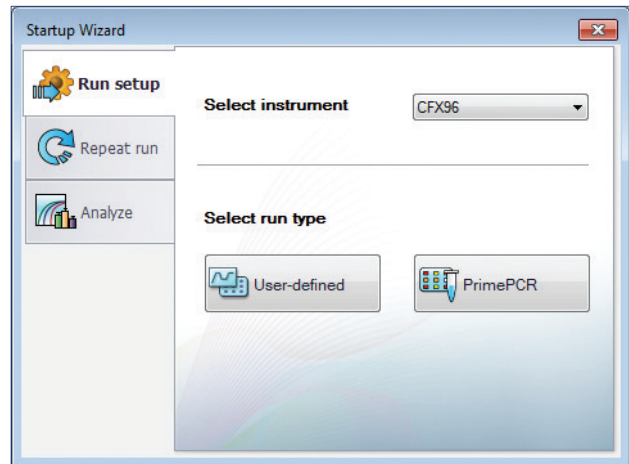


Fig. 4. Startup Wizard in CFX Maestro Software.

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	35
Annealing/extension	55°C	10 sec	35
	95°C	1 min	1
	55°C	1 min	1
Melt curve	70–95°C (in 0.2°C increments)	10 sec/step	1

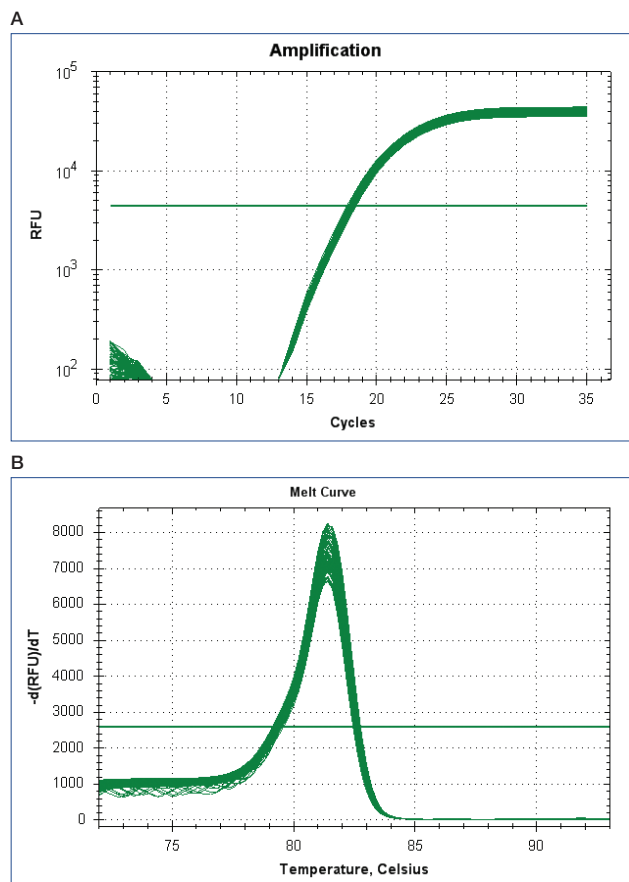


Fig. 5. Melt calibration data. A, amplification plot; B, melt curve plot with a single peak.

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 CFX Real-Time PCR System and analyzed using CFX Maestro Software. To open and generate a melt calibration file using Precision Melt Analysis Software:

1. Launch Precision Melt Analysis Software by double clicking the Precision Melt Analysis Software icon on the Desktop.
2. Click **Tools > Import Melt Calibration** from the Menu bar (Figure 6).
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.

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 3), 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 3), 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 each sample a cluster. 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 that of a reference curve.
4. Right click 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 the spreadsheet for options. Export the data in the spreadsheet by right clicking and selecting the **Export to Excel** option.

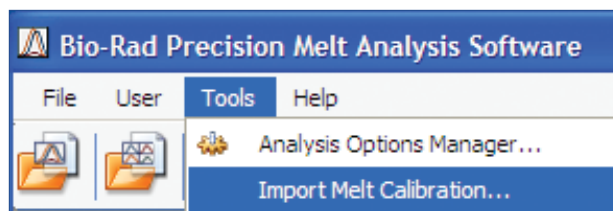


Fig. 6. Import Melt Calibration file.

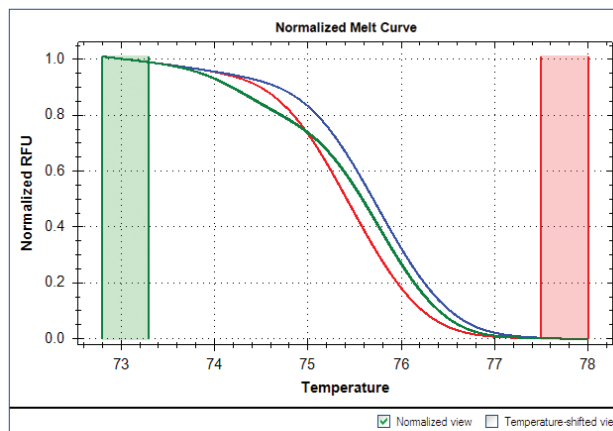


Fig. 7. Melt Curve pane.

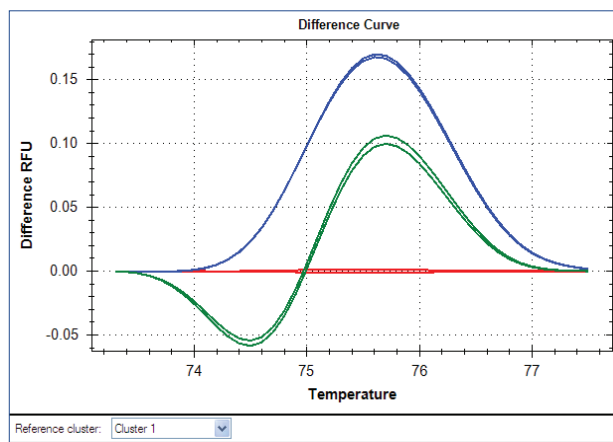


Fig. 8. Difference Curve pane.

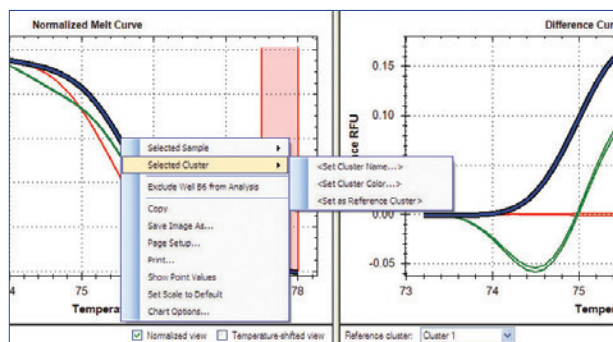


Fig. 9. Changing cluster parameters.

Viewing Well Group Data

Use the Well Group dropdown 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 any column header to sort rows based on that column.

Right click the spreadsheet and select **Sort** to sort rows based on multiple columns. Display data in the following ways:

- Right click the spreadsheet and select a format to export data as a Microsoft Excel, image, XML, or HTML file
- Select **Plate View** from the dropdown menu under the Precision Melt Data tab to display well data in a plate grid format
- Select **Charts** from the dropdown 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, which is displayed in the preview section (Figure 12), to include specific information in the report.
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.

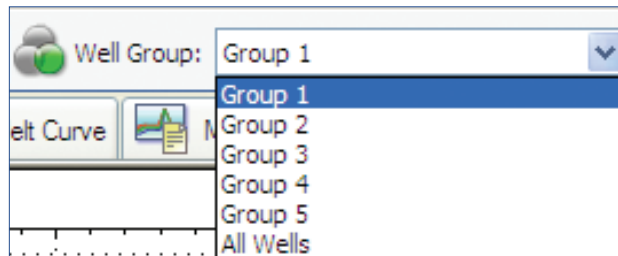


Fig. 10. Well Group selection.

Sample	Cluster	Percent Confidence	Call Type
A/t/t	Cluster 1	99.9	Auto
A/t/t	Cluster 1	99.9	Auto
A06 Unkn-08	H c/t	100.0	Auto
A07 Unkn-08	H c/t	100.0	Auto
B06 Unkn-09	C c/c	99.8	Auto
B07 Unkn-09	C c/c	99.9	Auto

Fig. 11. Precision Melt Data tab.

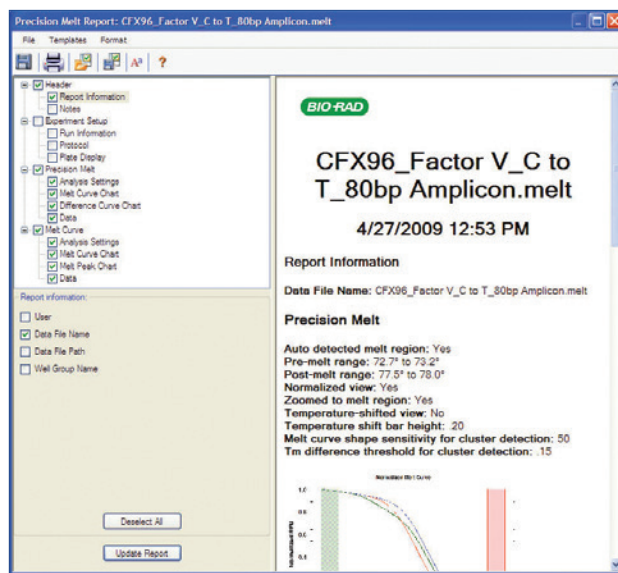


Fig. 12. Report window. Report options list features checkboxes for choosing information to include in a report.

Visit [bio-rad.com/PrecisionMeltAnalysisSoftware](https://www.bio-rad.com/PrecisionMeltAnalysisSoftware) for more information.

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