Melt Calibration Kit

Catalog #184-5020

Kit Contents

The following materials are provided in the melt calibration kit:

- Melt calibration DNA standard
- Melt calibration primers
- Precision melt supermix (catalog #172-5110)

Storage and Stability

Precision melt supermix is stable until the date indicated when stored in a constant temperature freezer at –20°C. For convenience, it may be refrozen for longer-term storage.

Product Overview

Before Precision Melt Analysis™ software can analyze data generated on a CFX96™, CFX96 Touch™, CFX384™, CFX384 Touch™, or CFX Connect™ real-time PCR detection system, a melt calibration must be performed. The calibration is required regardless of the intercalating dye that will be used in the experiments, including SYBR® Green I.

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, or CFX384 Touch system
- Hard-Shell® thin-wall 96-well skirted PCR plates with white shell and clear wells (catalog # HSP-9601) or white wells (catalog #HSP-9655) for use with a CFX96, CFX96 Touch, or CFX Connect system

Preparing the Melt Calibration Plate

1. Add the required volume of each component to an appropriately sized tube (Table 1).

Table 1. Reaction setup for a melt calibration plate.

Component	Volume for CFX96, CFX96 Touch, or CFX Connect System	Volume for CFX384 or CFX384 Touch System
Precision melt 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
Totals	**2,400 µl**	**4,500 µl**

2. Cap the tube and mix the reaction components gently by vortexing.
3. Briefly centrifuge the tube to remove air bubbles and collect contents at the bottom.
4. Add the appropriate volume of the mixture into each well of a reaction plate.
   - ❑ For a CFX96, CFX96 Touch, or CFX Connect system, add 20 µl of the reaction mix to each well of the 96-well plate
   - ❑ For a CFX384 or CFX384 Touch system, add 10 µl of the reaction mix to each well of the 384-well plate
5. Seal the reaction plate with Microseal 'B' adhesive film. Centrifuge the plate to move all the reaction components to the bottom of the wells.

Performing the Melt Calibration Experiment

The melt calibration experiment is run on a CFX96, CFX96 Touch, CFX384, CFX384 Touch, or CFX Connect system and analyzed using CFX Manager™ software.

To run the melt calibration and generate a melt calibration data file:

1. Turn on the CFX96, CFX384, or CFX Connect 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. Click **OK** to launch the Experiment Setup window.
4. In the Protocol tab, select **Create New** to open the Protocol Editor.
5. Create the following protocol (Table 2).

Table 2. Melt calibration PCR protocol.

Cycling Step	Temperature	Time	# of Cycles
Enzyme activation	98°C	2 min	1
Denaturation	98°C	5 sec	35
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 **Select Existing** > **Sample Files** > **Melt Calibration** Folder, select the appropriate plate name based on your instrument and plate type, and click **Open**.
   - ❑ For a CFX96 or CFX Connect 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 check box 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_today's date**.
14. When the melt calibration run is complete, the data file is automatically processed and opened by CFX Manager software. Check the data file to ensure all wells display a tight amplification and a single melt peak (Figure 1).

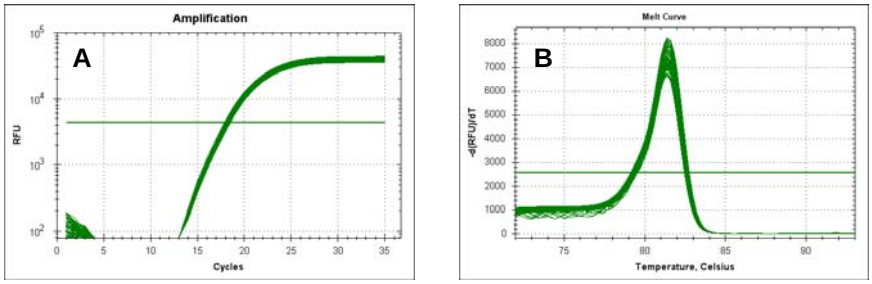

Fig. 1. Melt calibration data. A, amplification plot; B, melt curve plot with a single peak.

Importing a Melt Calibration File

The Precision Melt Analysis software is used to open data files that have been generated from an experiment performed on a CFX96, CFX96 Touch, CFX384, CFX384 Touch, or CFX Connect real-time PCR detection system and analyzed using CFX Manager software.

To open and generate a melt calibration file using the Precision Melt Analysis software:

1. Launch the 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.
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.

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