

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 μΙ	
Melt calibration DNA standard	120 μΙ	450 μΙ	
Melt calibration primers	14.4 μΙ	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.

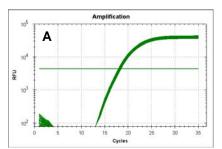
To run the melt calibration and generate a melt calibration data file:

- 1. Turn on the CFX96, CFX384, or CFX Connect system.
- 2. Double-click the CFX Manager software desktop icon to launch the software.
- 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).



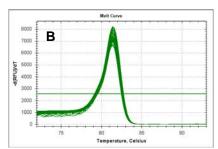


Fig. 1. Melt calibration data. A, amplification plot; B, melt curve plot with a single peak.

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.
- 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.

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following U.S. patents and corresponding patent claims outside the U.S.: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152 (claims 1 to 23 only), 5,733,258 (claims 1 and 6 only), 5,407,800, 5,322,770, 5,310,652, 5,994,056, 6,171,785, and claims outside the U.S. corresponding to U.S. Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as apparatus or system claims in U.S. Patent No. 6,814,934) and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, U.S.A.

Hard-Shell PCR plates are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Nos. 7,347,977, 6,340,589, 6,528,302.

Bio-Rad Laboratories, Inc. is licensed by Biotium Inc. to sell reagents containing EvaGreen dye for use in real-time PCR, for research purposes only.

SYBR® is a registered trademark of Molecular Probes, Inc. EvaGreen is a registered trademark of Biotium, Inc.