



LightCycler®

LightCycler® 480 Instrument Quick Guide



**For life science research use only.
Not for use in diagnostic procedures.**

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Revision History

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| Publication Reference Number | Date | Revision Purpose |
|-------------------------------------|---------------|-------------------------|
| 5125-00-1113 | November 2013 | New Document Creation |

General Required Materials

Standard Laboratory Equipment

| Product Name |
|---|
| Microwell Plate Centrifuge |
| Nuclease-free, aerosol-resistant pipette tips |
| Microcentrifuge and 1.5 mL microcentrifuge tubes |
| Micropipettes (P10 or P20, P200, P1000 or equivalent) |

Disposables

| Product Name | Catalog Number | Description |
|--|----------------|-----------------------------|
| LightCycler® 480 Multi-well Plate 96-well - White | 04 729 692 001 | 50 plates and sealing foils |
| LightCycler® 480 Multi-well Plate 384-well - White | 04 729 749 001 | 50 plates and sealing foils |
| LightCycler® 480 Multi-well Plate 96-well - Clear | 05 102 413 001 | 50 plates and sealing foils |
| LightCycler® 480 Multi-well Plate 384-well - Clear | 05 102 430 001 | 50 plates and sealing foils |
| LightCycler® 480 Sealing Foil | 04 729 757 001 | 50 sealing foils |

Reagents

| Reaction Type | Product Name | Catalog Number | Description |
|---|---|----------------|---|
| Reverse Transcription with RNase H activity | Transcriptor 1st Strand cDNA Synthesis Kit | 04 379 012 001 | For 50 (20 µL) reactions |
| SYBR Green Master | LightCycler® 480 SYBR Green I Master | 04 707 516 001 | 5 mL (5 x 20 µL) 2x concentrated Master Mix for 250 (20 µL) reactions |
| High Resolution Melting | LightCycler® 480 High Resolution Melting Master | 04 909 631 001 | 5 mL (5 x 1 mL) 2x concentrated 25 mL MgCl ₂ stock solution for 500 (20 µL) reactions |
| Hybridization Probes | LightCycler® 480 Genotyping Master | 04 707 524 001 | 1.5 mL 5x concentrated Master Mix for 250 (20 µL) reactions |
| Hydrolysis Probes <i>TaqMan® Probes or Universal Probe Library (UPL)</i> | LightCycler® 480 Probes Master | 04 707 494 001 | 5 mL (5 x 1 mL) 2x concentrated Master Mix for 250 (20 µL) reactions |
| | LightCycler® 480 Control Kit | 04 710 924 001 | Kit for quantitative Real-Time PCR and genotyping reactions |

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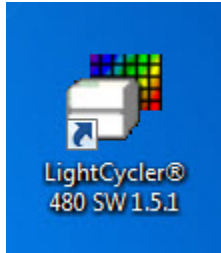
Getting Started

1. Log on to the LightCycler® computer.

Username: Operator

Password: LC480

2. Start the LightCycler® 480 software by double-clicking the <LightCycler480> icon.



3. Enter the username and assigned password to log in to the LightCycler® 480 software.

First Time Login (new database)

Username: admin

Password: LightCycler480

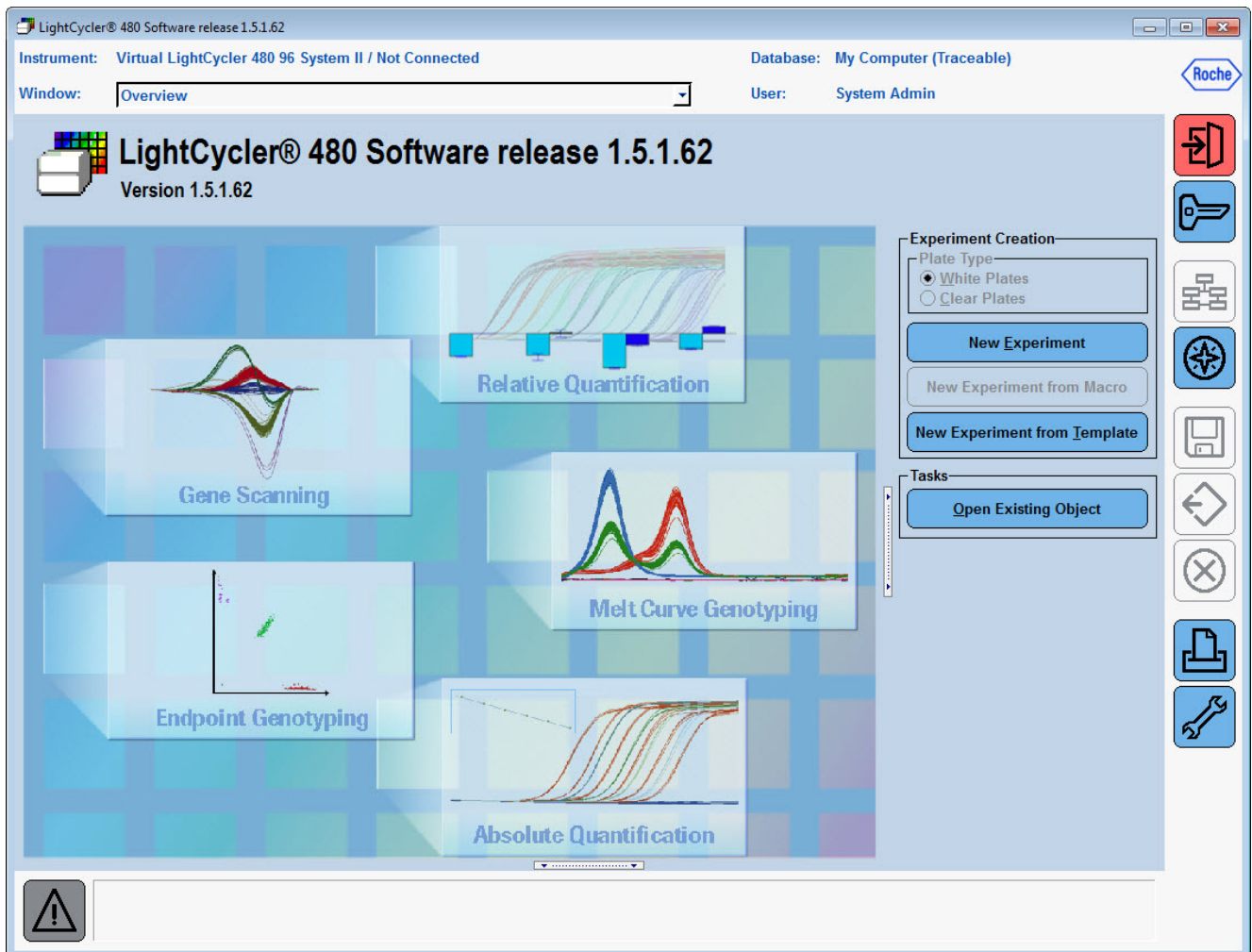
Normal Login

Username: admin

Password: Roche480

LightCycler® 480 Instrument Basics

4. The overview screen displays. This screen allows entry of a new experiment with or without use of a template for the conditions, or run a previously programed macro.



Software Navigation Overview



Exit the software



Logout of the current database



Switch to Overview menu



Display Navigator window



Save the current experiment



Export the current experiment to an “.ixo” file



Close the current experiment



Print the current screen



Display Tools window

Navigator window: displays the objects stored in the database as folders and files, allowing the user to work with objects in the database as well as import and export

Exporting as .ixo: creates a copy which can be imported to another computer or database

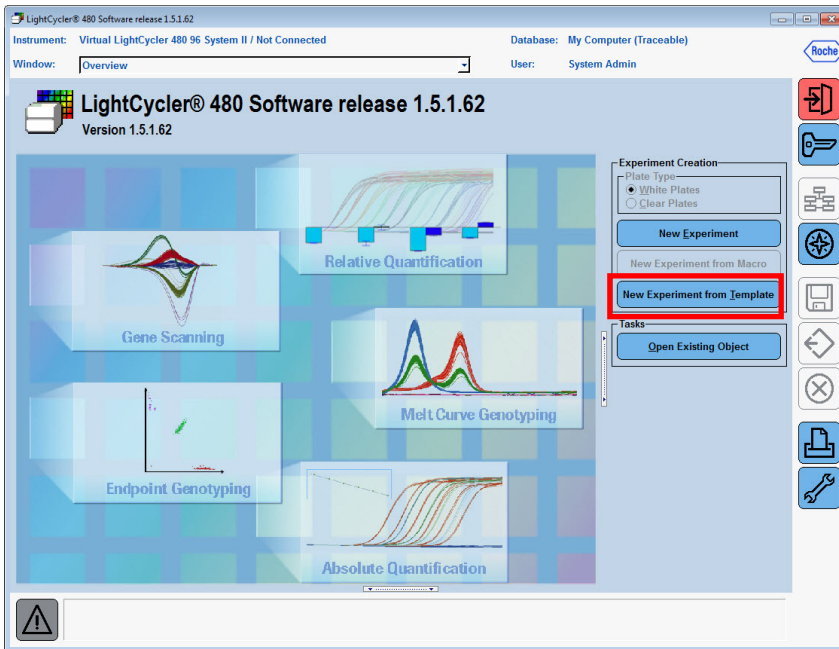
Tools window: allows user to change passwords, edit system settings, view database status and Error Log, define filter combination settings

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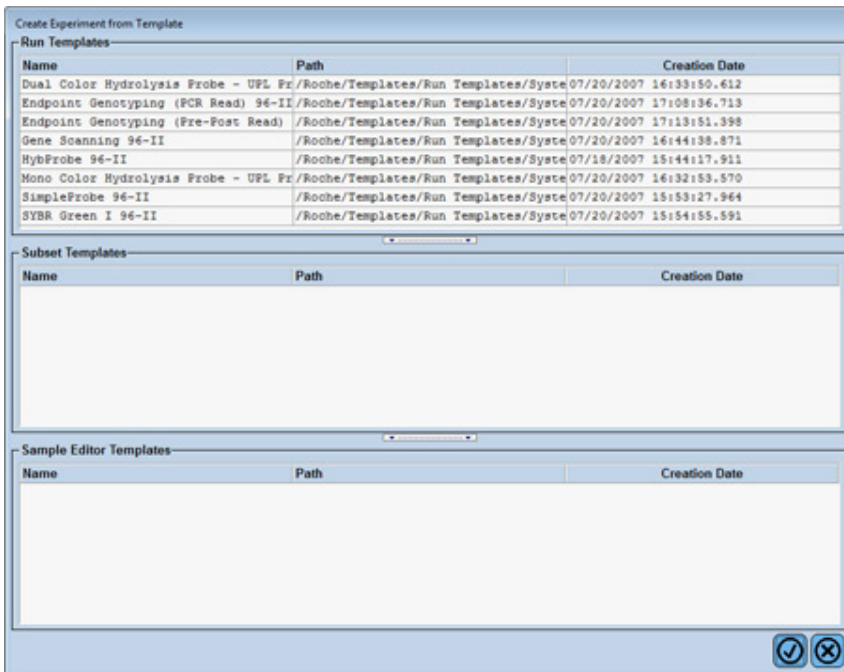
Create a New Experiment from Template

Templates provide a convenient way to speed up the process of creating an experiment. You can use Roche general templates for the different types of Real-time PCR reactions, as well as create your own templates based on the specific conditions of your experiments.


1. Click **New Experiment from Template** in the *Overview* window.

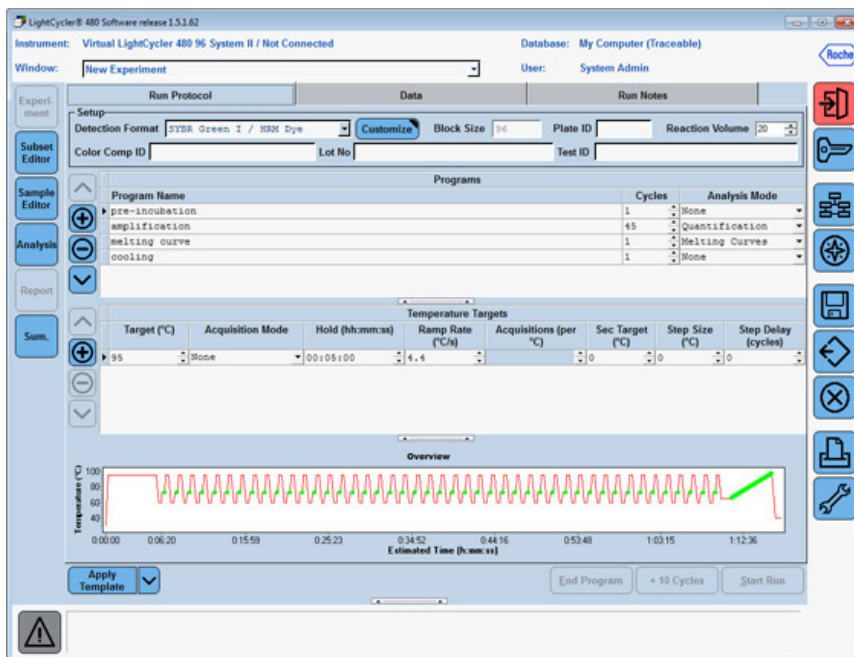


2. The *New Experiment from Template* window displays the templates that match the connected instrument.



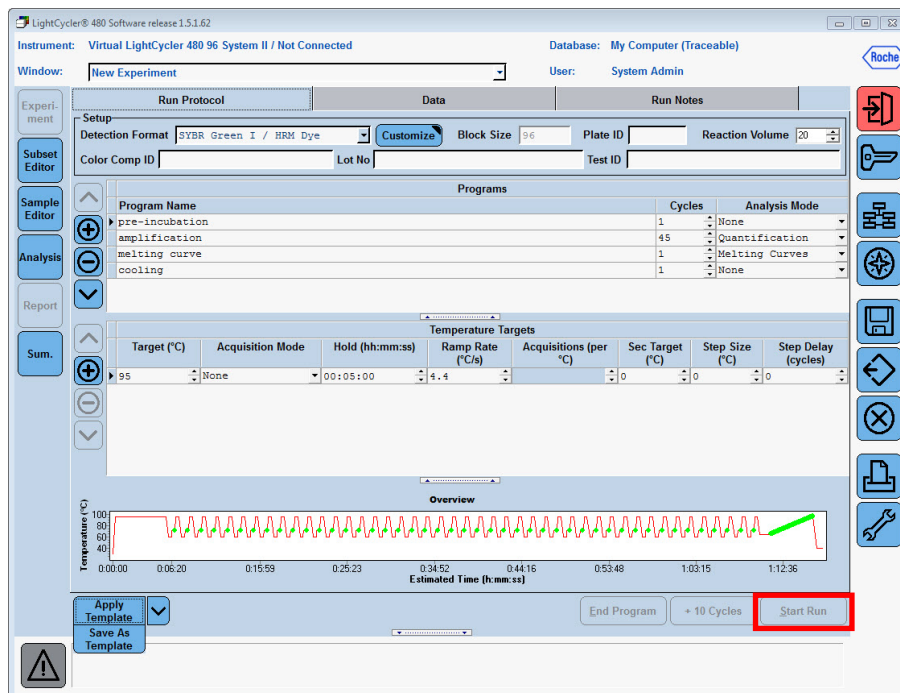
New Experiment from Template

3. Select a run template.
 - a. Dual Color Hydrolysis Probes/UPL Probe
 - duplex reactions using Hydrolysis Probes (UPL/TaqMan) labeled with FAM and VIC, HEX or Yellow 555
 - b. End-point Genotyping (PCR read)
 - genotyping with FAM and VIC or HEX-labeled Hydrolysis or TaqMan probes, PCR and data collection (Allelic discrimination)
 - c. End-point Genotyping (Pre-Post read)
 - genotyping with FAM and VIC or HEX-labeled probes, data collection only, PCR performed in another instrument (Allelic Discrimination)
 - d. Gene Scanning
 - basic protocol for High Resolution Melting experiments, including touch-down protocol
 - e. HybProbe
 - reactions using Hybridization Probes, labeled with Fluorescein and LightCycler Red 640
 - f. Mono Color Hydrolysis Probe/UPL
 - reactions with Hydrolysis Probes (UPL/TaqMan) labeled with FAM
 - g. Simple Probes
 - melting curve genotyping reactions using a single Fluorescein-labeled probe (Simple Probe)
 - h. SYBR Green
 - reactions using SYBR Green and similar double-strand-intercalating dyes, includes melting curve
4. Click . The selected run-template is applied.
5. Modify run conditions (reaction volume, number of cycles, target temperature, hold, ramp rate, etc.) as needed.



New Experiment from Template

- The “Start Run” icon becomes active once the multi-well plate is loaded in the instrument.



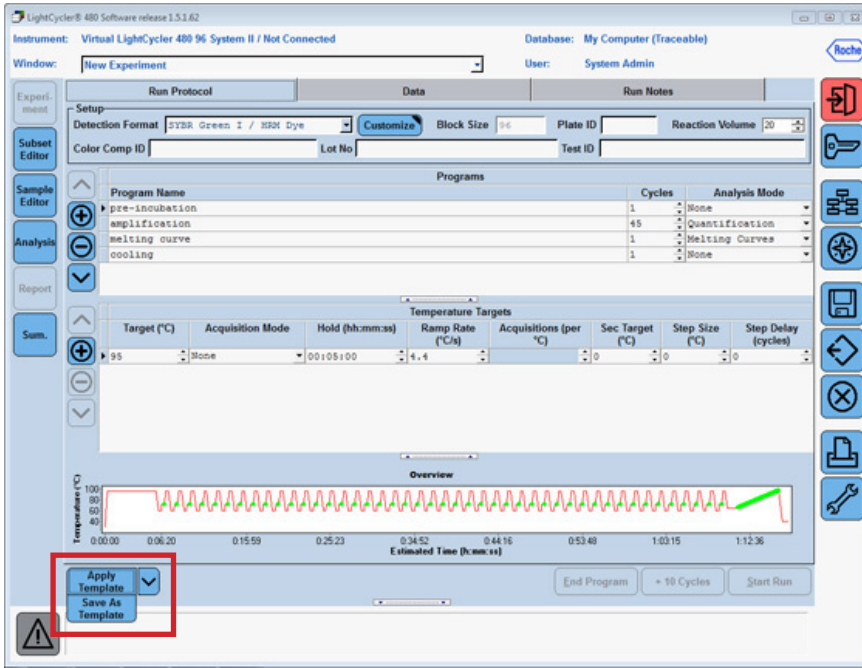
- Click **Start Run**.
- Enter the experiment name in the dialog box.

New Experiment from Template

Save Run Conditions as a Template


If you modify the run conditions, you can save it as a new template for future use.

1. Open or create the run to be used as a template.
2. Click the drop-down next to “Apply Template” and select **Save as Template**.



3. Name the run template and select a location to save the template.

Note: The default storage location for templates is the user's Template folder.

4. Click . The template is saved and will appear in the *New Experiment from Template* window in the future.

Typical SYBR Green Reaction Protocol

Reaction Setup

| <i>Reagent</i> | <i>Final Concentration</i> | <i>Volume (μL) / one reaction</i> |
|---|----------------------------|-----------------------------------|
| Water | | 1.0 |
| Primer - Forward (5 μM Stock) | 0.5 μM | 2.0 |
| Primer - Reverse (5 μM Stock) | 0.5 μM | 2.0 |
| LightCycler® 480 SYBR Green I Master | 1x | 10.0 |
| | Total | 15.0 |
| Add 5 μL template DNA for a total volume of 20 μL | | |

Run Program

| Program Name | Cycles | Analysis Mode | |
|---------------------|-------------------------|------------------------|------------------------------|
| Pre-incubation | 1 | None | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | |
| 95 | None | 00:05:00 | |
| <hr/> | | | |
| Program Name | Cycles | Analysis Mode | |
| Amplification | 45 | Quantification | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | |
| 95 | None | 00:00:10 | |
| 60 | None | 00:00:10 | |
| 72 | Single | 00:00:10 | |
| <hr/> | | | |
| Program Name | Cycles | Analysis Mode | |
| Melting Curve | 1 | Melting Curves | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | <i>Acquisitions (per °C)</i> |
| 95 | None | 00:00:05 | |
| 65 | None | 00:01:00 | |
| 97 | Continuous | | 5 |
| <hr/> | | | |
| Program Name | Cycles | Analysis Mode | |
| Cooling | 1 | None | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | |
| 40 | None | 00:00:30 | |

Basic Real-Time Chemistry Protocols

Typical Mono Color Hydrolysis Probe (*TaqMan*[®]/UPL) Reaction Protocol

Reaction Setup

| <i>Reagent</i> | <i>Final Concentration</i> | <i>Volume (μL) / one reaction</i> |
|---|----------------------------|-----------------------------------|
| Water | | 0.6 |
| Primer - Forward (5 μM Stock) | 0.5 μM | 2.0 |
| Primer - Reverse (5 μM Stock) | 0.5 μM | 2.0 |
| Probe (10 μM Stock) | 0.2 μM | 0.4 |
| LightCycler [®] 480 Probe Master | 1x | 10.0 |
| | Total | 15.0 |

Add 5 μL template DNA for a total volume of 20 μL

Run Program

| Program Name | Cycles | Analysis Mode |
|---------------------|-------------------------|------------------------|
| Pre-incubation | 1 | None |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> |
| 95 | None | 00:10:00 |

| Program Name | Cycles | Analysis Mode |
|---------------------|-------------------------|------------------------|
| Amplification | 45 | Quantification |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> |
| 95 | None | 00:00:10 |
| 60 | Single | 00:00:30 |
| 72 | None | 00:00:01 |

| Program Name | Cycles | Analysis Mode |
|---------------------|-------------------------|------------------------|
| Cooling | 1 | None |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> |
| 40 | None | 00:00:30 |

Typical Hybridization Probe (HybProbe) Reaction Protocol

Reaction Setup

| <i>Reagent</i> | <i>Final Concentration</i> | <i>Volume (μL) / one reaction</i> |
|---|----------------------------|-----------------------------------|
| Water | | 6.2 |
| Primer - Forward (5 μM Stock) | 0.5 μM | 2.0 |
| Primer - Reverse (5 μM Stock) | 0.5 μM | 2.0 |
| Fluorescein Probe (10 μM Stock) | 0.2 μM | 0.4 |
| Red Fluor Probe (10 μM Stock) | 0.2 μM | 0.4 |
| LightCycler® 480 Genotyping Master | 1x | 4.0 |
| | Total | 15.0 |
| Add 5 μL template DNA for a total volume of 20 μL | | |

Run Program

| Program Name | Cycles | Analysis Mode | |
|---------------------|-------------------------|------------------------|------------------------------|
| Pre-incubation | 1 | None | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | |
| 95 | None | 00:10:00 | |
| <hr/> | | | |
| Program Name | Cycles | Analysis Mode | |
| Amplification | 45 | Quantification | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | |
| 95 | None | 00:00:10 | |
| 60 | Single | 00:00:10 | |
| 72 | None | 00:00:10 | |
| <hr/> | | | |
| Program Name | Cycles | Analysis Mode | |
| Melting Curve | 1 | Melting Curves | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | <i>Acquisitions (per °C)</i> |
| 95 | None | 00:01:00 | |
| 40 | None | 00:02:00 | |
| 95 | Continuous | | 5 |
| <hr/> | | | |
| Program Name | Cycles | Analysis Mode | |
| Cooling | 1 | None | |
| <i>Target (°C)</i> | <i>Acquisition Mode</i> | <i>Hold (hh:mm:ss)</i> | |
| 40 | None | 00:00:30 | |

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