protocol v1.04

7900HT qPCR instrument (Applied Biosystems) quick start guide

Important notes

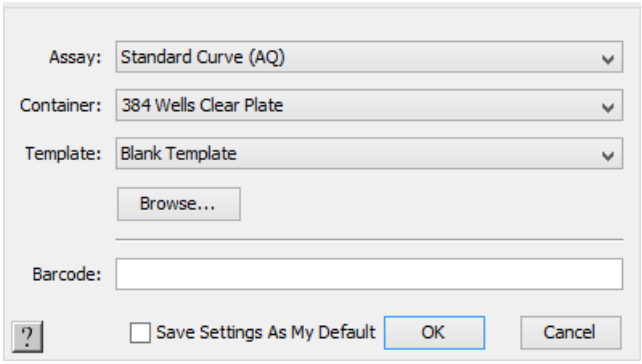
- This document is only a quick start guide, please refer to the official instrument user manual for *in depth* instructions. Contact us if you have questions or need help with troubleshooting.
- Check the compatibility of your mastermix with this instrument. In particular, mastermixes containing “high SYBR concentration” should not be used (for instance SYBR SELECT from Applied Biosystems). In addition, mastermixes containing « high rox » concentrations are preferred over “low rox” ones.

  Note: Many mastermixes contain a passive reference (most often ROX), which is a dye not involved in the qPCR reaction itself but used to normalize for putative volume differences between wells. If you don’t know if your mastermix includes ROX or its concentration, please check your mastermix user guide.

- Booking is done through the BBS GECF portal. Pricing is available on GECF web site.

How to start the run

- Switch on the instrument if needed. If the instrument was already on, make sure no run is still ongoing.
- Open the SDS software.
- Click on the “New documents” button, and set the options this way:
  - Assay: the default setting (Standard curve) is the one providing the broadest compatibility with downstream analysis software, so we recommend keeping it as such.
  - Container: select the relevant plate type.
  - Template: choose blank template if you will use the txt file from GECF Hamilton robot.



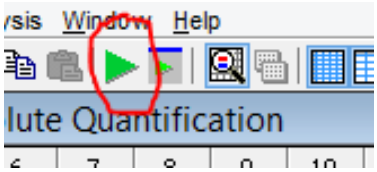
- File →“import” → select the txt file created by the Hamilton robot. Avoid non-standard characters in samples and detectors names.
- Save the file into your user/lab folder.
- If you are using Rox-containing mix (highly recommended), check Rox is selected at the bottom of the setup page. If unsure, refer to the user manual of your mastermix, or select it anyway as it can then be unchecked later after the run.

- In the “Instrument” tab, “Thermal profile” sub-tab:
    - Edit the thermal profile if needed (refer to your mastermix user manual). *Do not use fast settings unless you are fully aware of the particularities of this method (not recommended).*
    - On the upper right corner, change reaction volume to 10ul if your 384-well plate was assembled by the Hamilton robot (15ul for Hamilton-generated 96-well plate).
    - If using SYBR green, click on the last stage and click on “Add dissociation stage”.
- In the “real-time” sub-tab, click on “connect to the instrument”.
- “Open”, and insert your plate inside the instrument (make sure the A1 well is positioned according to the label on the plate holder). If the tray came out empty, make sure a plate is not stuck inside. Then “Close”.
- Click on the “run” button. *It is normal that the door opens and closes again at this stage.*
- *Note: For 96w plate, you have to change the block. Follow the instructions present next to the machine, and don’t forget to insert back the default 384w block exactly at the end of your run. Make sure the block matches your 96w plate type (96w fast block for 96w fast plates). For the 96w block only, make sure you add the compression pad.*

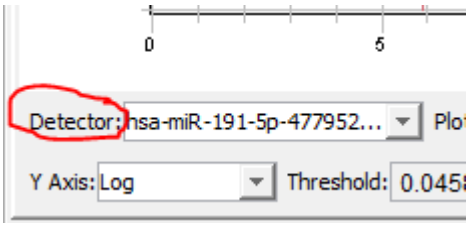
After the run

Analyzing the run

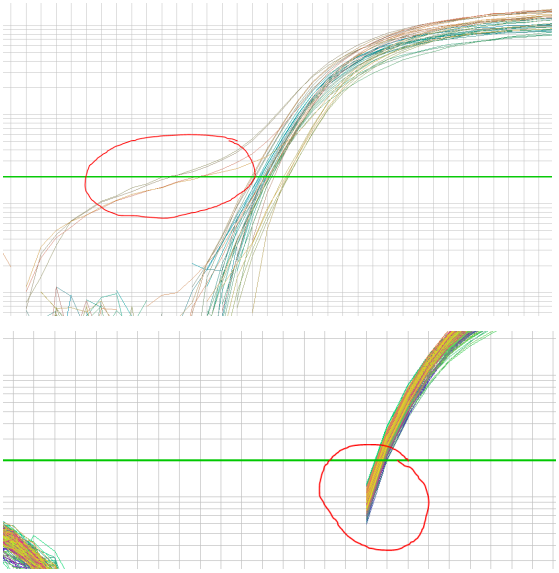
- Make sure you don’t perform the analysis while another run is ongoing (for instance the run of the next user). *If needed, we can provide you with the installation file for the SDS software (PC only).*
- Click on the “Analyze” button (large green triangle) to automatically set the threshold and background and calculate the Ct’s of your samples.



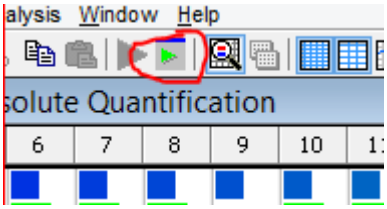
- Inspect the amplification plot for putative issues, by selecting the samples in the table, and the detector/assay/gene in the drop-down list below the amplification plot:



You should check for each gene that the threshold and baseline were correctly assessed: 1) Check that the automatic threshold is in the exponential part of the amplification curve (appearing linear due to log scale); 2) Check that the baseline is correctly set by checking for absence of aberrant behavior of the beginning of the curves, such as the ones depicted below:



- In case the automatically-set threshold and baseline need to be manually adjusted, press on the little green triangle (“analysis settings”) to adjust parameters manually (ask us for help if needed).

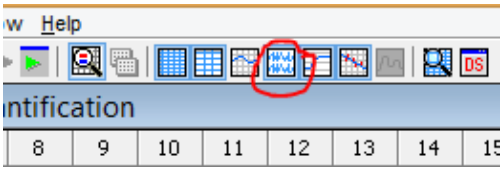


Melting curve analysis

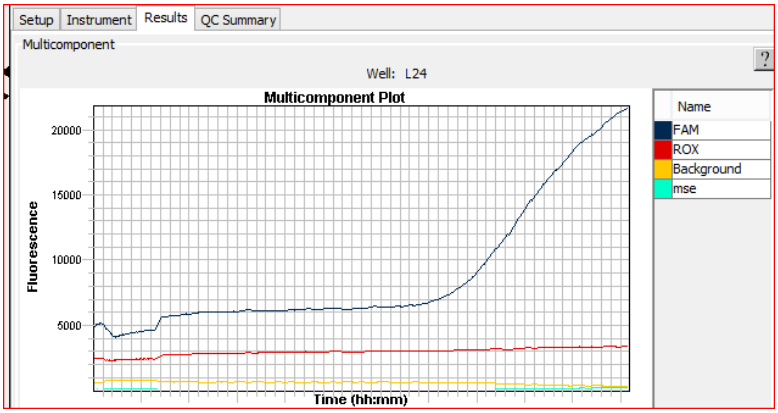
If you used SYBR green, review the melting curves (dissociating curve tab) for absence of multiple peaks. Typical peaks will appear between approximately 77° and 88°. Secondary peaks lower than that may represent primer dimers, and higher may represent genomic DNA contamination (amplicon containing an intron).

Detecting issues

- The software automatically detects issues with the amplification and label the affected samples with a yellow triangle in the table. In addition a summary of all the detected issues is found in the “QC summary” tab.
- For troubleshooting purpose, the “multicomponent plot” view can be useful since it shows raw signal for all relevant channels (FAM/SYBR, rox, background). You can select it here:



The plot looks like this:



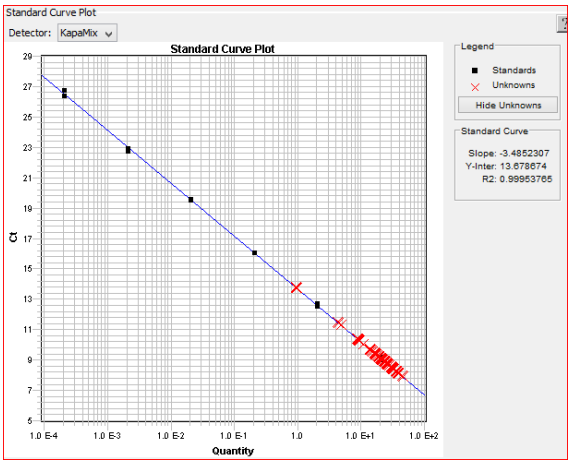
Exporting data

- File → export. The txt file can then be imported into excel and most commercial analysis software.

Data analysis

Absolute quantification analysis

- If your experiment includes a standard curve for absolute quantification of samples, it will be automatically calculated by the software and will appear in the results tab. Make sure your samples are within the range of the standard curve, and that the curve linear regression fits well enough the standard curve points (black dots):



Relative quantification analysis (delta delta Ct)

- The SDS software can automatically calculate the delta delta Ct for relative quantification of gene expression. Most people use dedicated macros and software, but if you want to perform this analysis in SDS first convert the file into a relative quantification file by selecting "Tools" → "AQ RQ converter". After conversion, the exported txt file is much less broadly recognized by third party macros and software, therefore we recommend exporting a standard version of the Cts data before the conversion.

- Accurately using the delta delta Ct method requires that all your primer couples have a similar efficiency. A serial dilution of a cDNA sample or plasmid should be performed to calculate these efficiencies.

- Contact us in case you need some help for the delta delta Ct analysis. We for instance provide access to the commercial Genex software, which allows selecting several housekeeping genes for normalization.

Versions log

- v1.01: initial release.

- v1.02: minor revisions. Indicated not to perform an analysis while a run is ongoing.

- v1.03: indicated to check no plate is stuck in the instrument when the plate holder goes out empty. Clarified how to tweak analysis settings for baseline issues.

- v1.04: Clarified to avoid non-standard characters in samples/detectors names. Clarified how to book and where to find prices.