LightCycler® 480 Instrument Absolute Quantification Quick Reference Card

Real-Time quantitative PCR data are collected and analyzed using the LightCycler® 480 software analysis modules:

- Absolute Quantification/Second Derivative Maximum
- Absolute Quantification/Fit Points Method
- Relative Quantification - (Delta Delta CT Method or Efficiency-Corrected Calibrator Normalized)
- Tm Calling
- Gene Scanning
- Melt Curve Genotyping
- End Point Genotyping
- Color Compensation.

Absolute Quantification Analysis

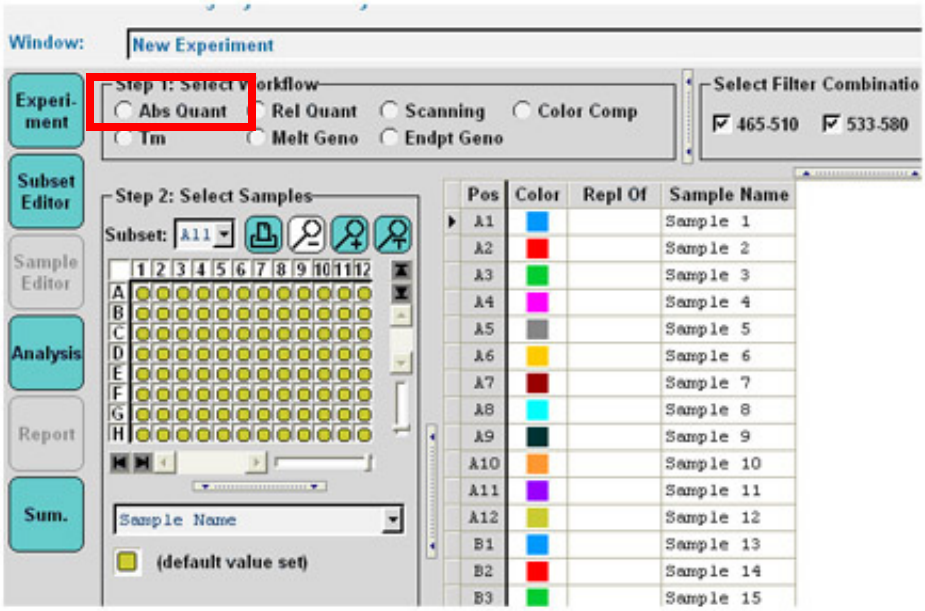
Sample concentrations are extrapolated from a standard curve created with standard samples along a concentration gradient with specific concentration values assigned. The standard curve can be included within the run (internal standard curve) or could have been generated in a previous run (external standard curve).

Important: *To use an external standard curve imported from a previous run, the software needs a reference point included in the current run. One concentration standard must be on every run to align the standard curve into the new run. The software shifts the imported standard curve based on how the reference standard compares to the previously run concentration.*

Crossing point values (Cp is equivalent to Ct) are used to determine unknown sample concentrations from a standard curve.

Performing an Absolute Quantification Analysis

1. Open the experiment you want to analyze in the main window.
   Note: *If the experiment completed the run, it remains open and ready to analyze. Previous experiments are located in the* ***Experiments*** *folder of the Navigator.*
2. If sample information was entered during run setup, proceed to step 5; otherwise, click the **Sample Editor** object on the left side of the active display.
3. In the Sample Editor, select the **Abs Quant** workflow.

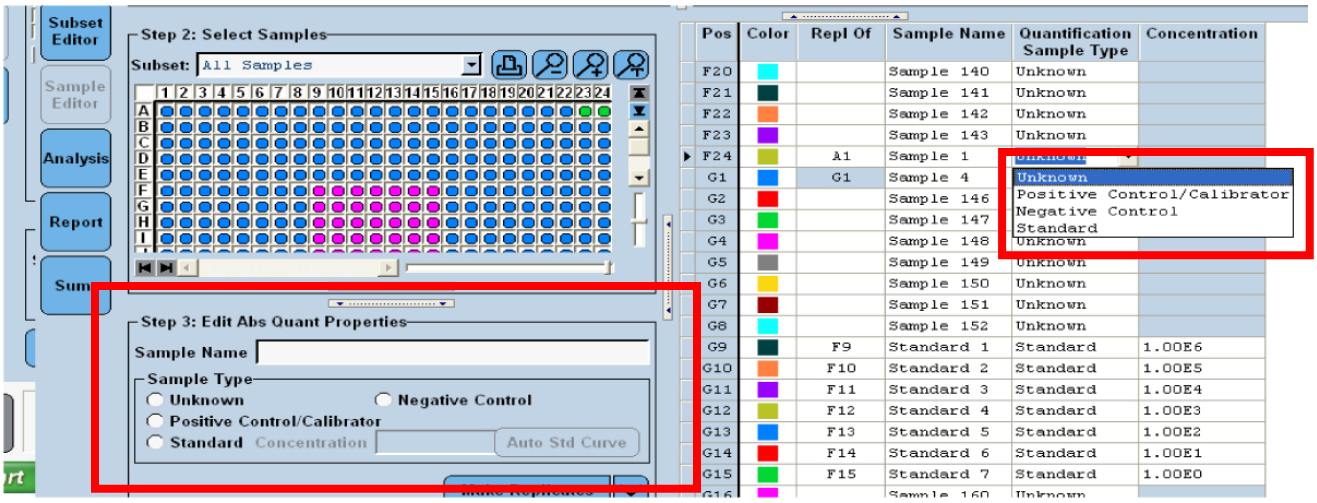


LightCycler® 480 Instrument Absolute Quantification Quick Reference Card

Absolute Quantification Analysis, continued

Performing an Absolute Quantification Analysis, continued

4. Enter sample information related to the absolute quantification analysis (identify samples as “Standard” or “Unknown” samples and enter the appropriate concentration estimates for standards which are used to create the standard curve.



5. Click **Analysis** on the module bar, located on the left side, to go into the analysis module of the software.
6. Select the analysis you want to create from the analysis list:
   - Absolute quantification/2nd derivative Max
   - Absolute quantification/Fit Points.

Important: ***How to determine the crossing point (two algorithms are available to determine the crossing point):***

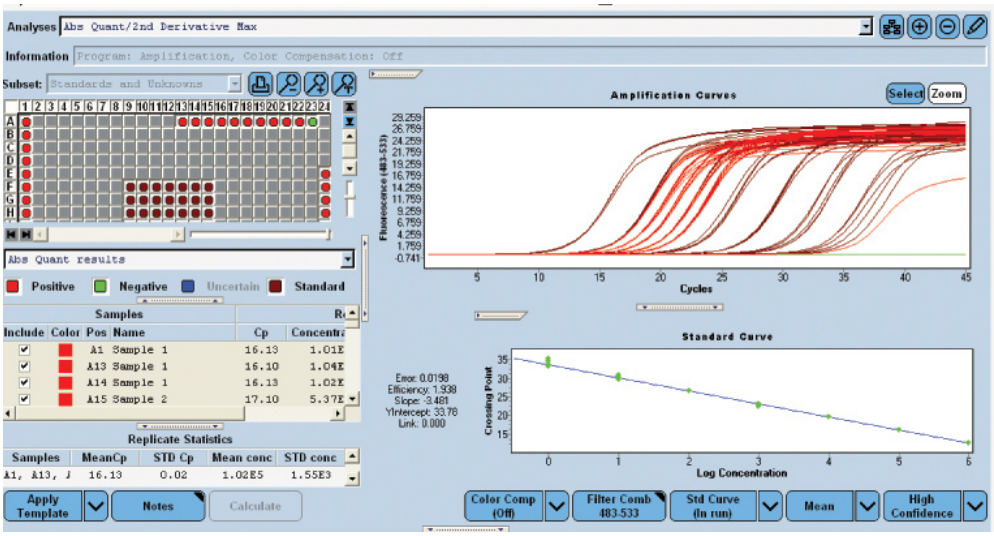
- *Automated second derivative method: This method does not require user input*
- *Fit points method: This method allows the user to set a threshold line. The position where the log-linear curve crosses the threshold line becomes the crossing point.*

7. In the Create New Analysis dialog box, select an analysis subset and Quantification program (selected by default), click **OK**.
8. If a standard curve was included in the experiment, select **std curve (in run),** or, if the standard curve was not included in the experiment, browse to select **std curve external**.
   - By default, all samples are included in result
   - Double-click the check box next to the sample or press the <space> bar to exclude a specific sample from the analysis.

LightCycler® 480 Instrument Absolute Quantification Quick Reference Card

Absolute Quantification Analysis, continued

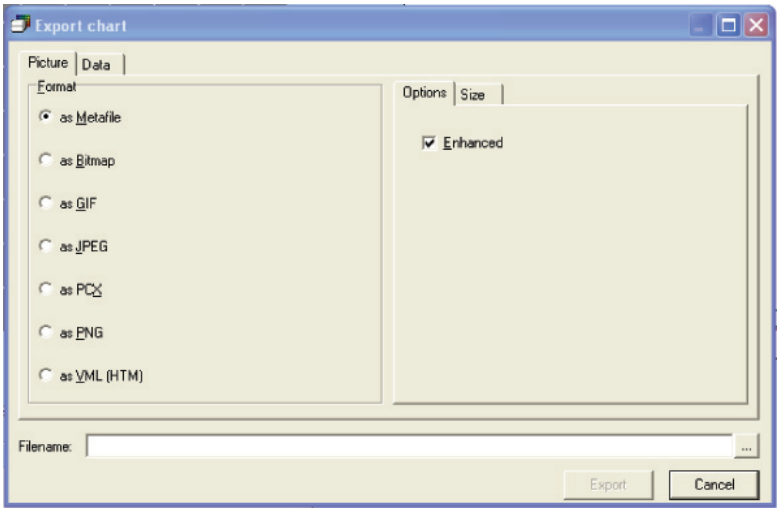
9. Click **Calculate**.



- To view the amplification curves for one or more samples, highlight the sample names in the sample list or click the corresponding well in the plate picture.

Important: ***External Standard Curve*** *- the external standard curve must be from an experiment with the same detection format, filter combination, and color compensation settings as the current experiment. The external curve and current experiments can be generated on different blocks (96 or 384). If using an external standard,* ***you must include one of the Standard concentrations in the new experiment as a reference****. The software calculates the concentration for each sample based on where each sample crossing point correlates to the Standard Curve.*

10. To export individual data figures or tables, right-click within the information section you want to export; then, choose the external format to use. Click the "**...**" button to browse to the location to save the file and assign a name to your export file. Click **Export**.



LightCycler® 480 Instrument Absolute Quantification Quick Reference Card

Absolute Quantification Analysis, continued

11. Export the entire experiment through the Navigator using the Export button. Route to the appropriate drive/folder where saving. The complete file experiment can be imported into another computer with the same release of LightCycler® 480 software.

LightCycler® 480 Instrument Reagents and Disposables

Product Name - Reagents	Catalog Number	Pack Size/Description
LightCycler® 480 SYBR Green I Master	04 707 516 001	5 mL (5 x 1 mL)
LightCycler® 480 Probes Master	04 707 494 001	5 mL (5 x 1 mL)
LightCycler® 480 Genotyping Master	04 707 524 001	Master Mix, 5 x concentration, 4 x 384 µL, ready-to-use hot start multiplex PCR reaction mix containing a modified *Taq*® DNA polymerase, reaction buffer, dNTP mix (with UTP instead of dTTP), and 15 mM $MgCl_2$
LightCycler® 480 Control Kit	04 710 924 001	Kit for quantitative Real-Time PCR and genotyping control reactions using the LightCycler® 480 instrument.

Product Name - Disposables	Catalog Number	Pack Size/Description
LightCycler® 480 Multi-well Plate 96	04 729 692 001	5 x 10 plates and sealing foils
LightCycler® 480 Multi-well Plate 384	04 729 749 001	5 x 10 plates and sealing foils
LightCycler® 480 Sealing Foil	04 729 757 001	50 foils

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