Absolute Quantification Analysis

Sample concentrations are calculated based on a standard curve created with standard samples along a concentration range with specific assigned values. The standard curve can be included within the run (internal standard curve) or 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.*
>
> *For more information, please consult Chapter D, Section 4.2.3 (pages 164-166) of the LightCycler® 480 Operator's Manual.*

Crossing point (Cp) 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:** *Once the experimental run has completed, 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 **Sample Editor** on the left side of the active display.

3. In *Sample Editor > Step 1: Select Workflow*, select **AbsQuant** workflow.
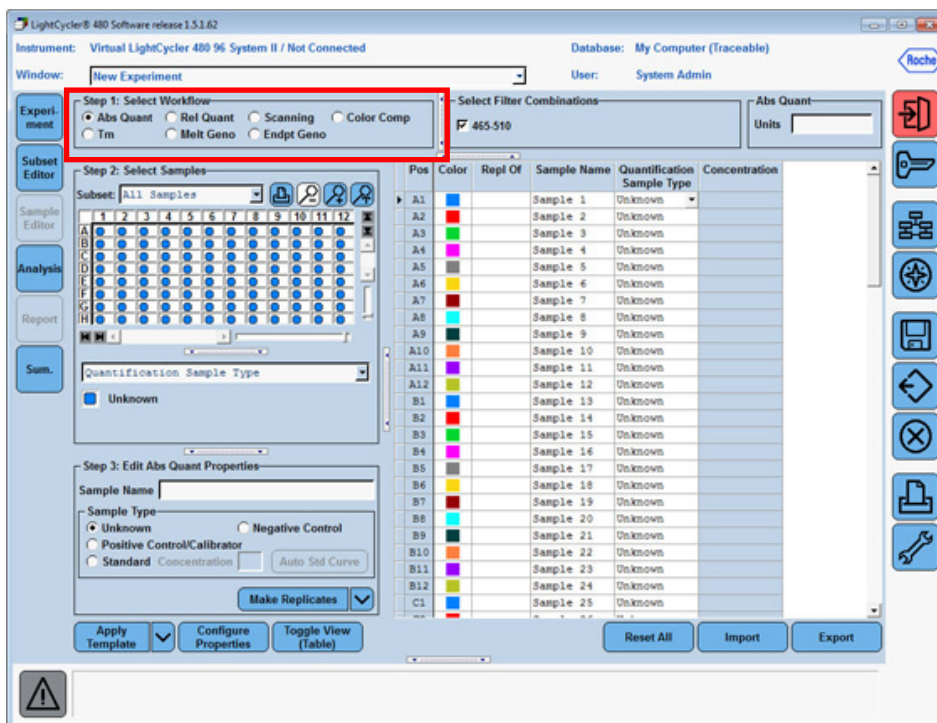
Absolute Quantification

4. Enter Sample information

    a. In *Sample Editor* > **Step 2: Select Samples**, select wells.

    b. In *Sample Editor* > **Step 3: Edit Abs Quant Properties,** enter the appropriate sample information for the selected wells. Identify samples as "*Unknown*" or "*Standard*" samples, entering the appropriate concentration for each standard.

    c. For reactions in replicate, select all the wells and click "**Make Replicates.**"
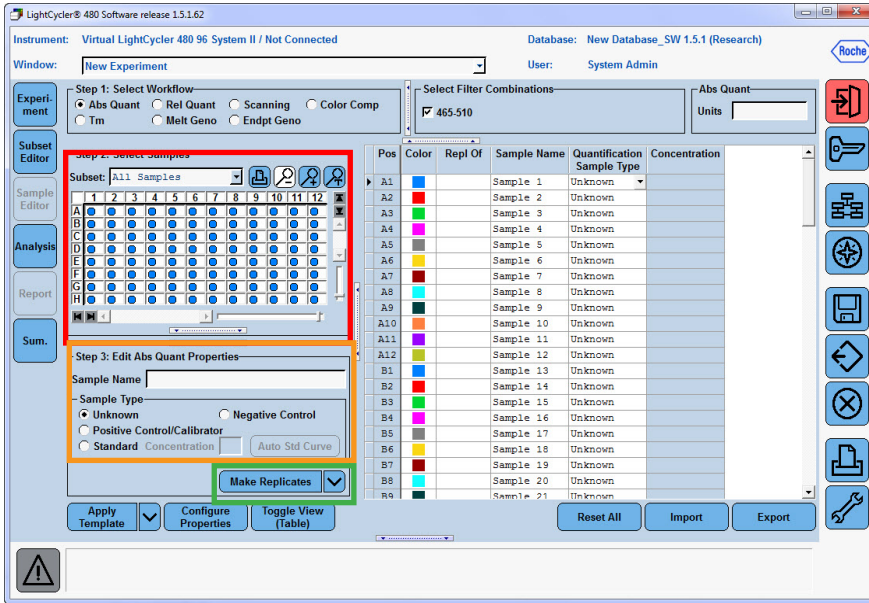
5. Click **Analysis**.
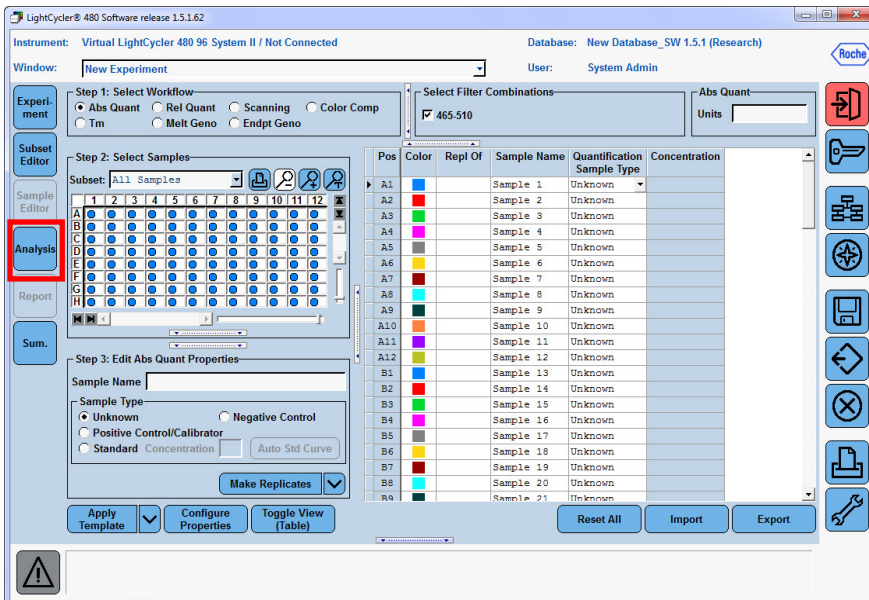
6. Select an analysis to create from the list.

   Important: *Two algorithms are available to determine the crossing point:*

   - Absolute quantification/2nd derivative max
     - automated, user input not required
     - the point at which the maximum change in slope of the amplification curve becomes the crossing point
   - Absolute quantification/Fit Points
     - user must set a threshold line
     - the point at which 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) and click **OK**.

8. If a standard curve was included in the experiment, select **std curve (in run)** from the drop-down menu. If an external standard curve needs to be imported, select **std curve external**.

   - By default, all samples are included in results
   - Double-click the check box next to the sample, or press the <space bar> to exclude a specific sample from the analysis

     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 Curve,* **you must include at least one of the Standard concentrations in the new experiment as a standard reference point for calculation**.

     For more information, please consult Chapter D, Section 4.2.3 (pages 164-166) of the LightCycler® 480 Operator's Manual.
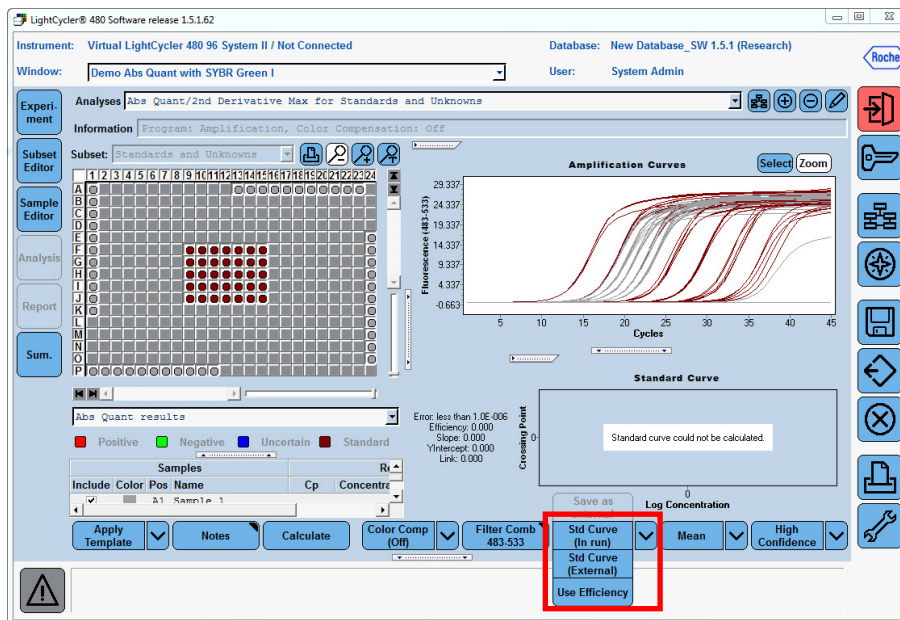
Absolute Quantification

9. Click **Calculate**.
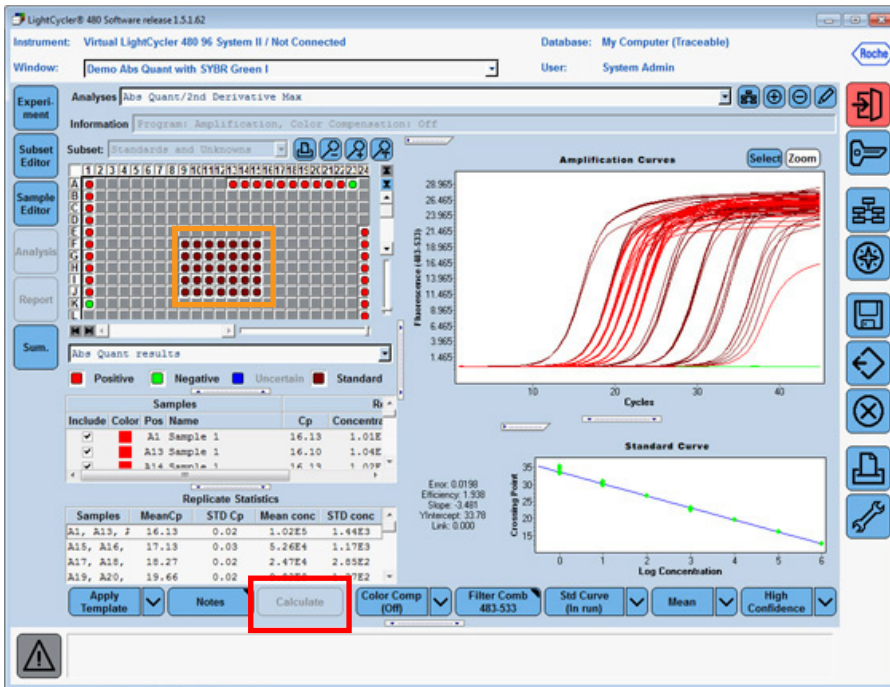
- To view the amplification curves for one or more samples, **highlight** the sample names in the sample list or click the corresponding well in the plate diagram.

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 **browse** (**...**) button to browse to the location to save the file and assign a name to your export file. Click **Export**.
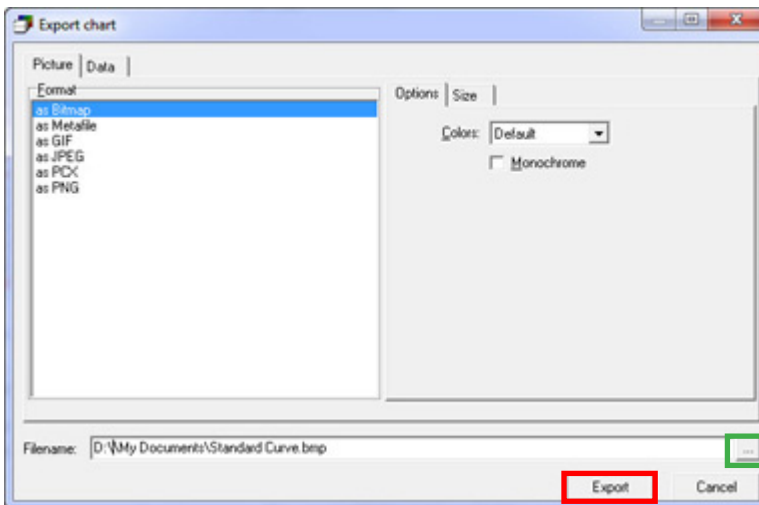
11. Export the entire experiment through the Navigator using the *Export* button. Map to the appropriate drive/folder for saving. The complete file experiment can be imported into another computer with the same release of LightCycler® 480 software.