

Standard Curve Analysis Module

USER GUIDE

for use with:

Diomni™ Design and Analysis (RUO) Software 3

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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The information in this guide is subject to change without notice.

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About the Standard Curve Analysis Module

The Standard Curve Analysis Module for Diomni™ Design and Analysis (RUO) Software 3 is used to determine absolute target quantity in test samples.

For more information about standard curve analysis, see Chapter 5, “About standard curve analysis”.



Workflow: Standard curve analysis

①	Set up a plate file
	Select a system template or existing plate file to set up a new plate file (page 8)
	Confirm or edit the run method (page 9)
	Confirm or edit the plate setup (page 9)
	Review and save the plate file (page 13)

②

Perform standard curve analysis

Review results in the Amplification Plot (page 15)

Select the Standard Curve Analysis Module (page 15)

Review results in the Standard Curve Plot (page 15)

Identify and omit outliers from standard curve analysis (page 16)

(Optional) Review dye signal profile in the Multicomponent Plot (page 16)

(Optional) Review signal profile in the Raw Data Plot (page 17)

(Optional) Edit standard curve analysis settings

For detailed instructions about setting up a plate file, see the primary user guides for the software. See “Related documentation” on page 22.

Select a system template or existing plate file to set up a new plate file


A plate file contains the information that is necessary to perform an instrument run, including instrument setup, run method, plate setup, and analysis setting.

A system template is a non-editable plate file that is included with the software.

A new plate file must be created from a system template or a previously created plate file.

For detailed information about system templates and plate files, see the primary user guides for the software.

IMPORTANT! Select a system template or a plate file that corresponds to your instrument, block, and run mode. These properties are not editable after the plate file has been created.

1. Click  **Run templates**.
The **Plate Gallery** is displayed.
2. Click the tab at the top of the **Plate Gallery** that is associated with the type of plate file to be used to set up a new plate file.

Tab	Description
Recents tab	Contains plate files that were recently opened. Recently opened plate files from the System Templates tab and the My Plate Files tab do not populate this tab. Click a plate file to open it. The plate file can be edited, then saved, or saved as a new plate file.
My Plate Files tab	Contains plate files that were previously saved to the My Plate Files tab. Click a plate file to open it. The plate file can be edited, then saved, or saved as a new plate file.
System Templates tab	Contains system templates. System templates are non-editable plate files that are included with the software. Click a system template to automatically generate a new plate file that can be edited, then saved.

3. In the left pane, select the appropriate options to filter the system template and plate file lists.

- **Instrument**
- **Block**
- **Run Mode**
- **Analysis**

Note: Thermal protocol, plate setup, and post-run analysis options are independent of analysis module selection. Analysis module selection can be changed at any point during plate file setup or post-run analysis (see “Select the Standard Curve Analysis Module” on page 15).

4. Open the plate file.

- Hover over the plate file, then click ... **(More Options)**.
The ... **(More Options)** menu for the plate file displays all of the options to open the plate file.
- Click the plate file.

The plate file opens in the **Run Method** tab.

Confirm or edit the run method

For most analysis, the default run method is appropriate. The following options are compatible:

- PCR
- 1-step RT-PCR
- 2-step RT-PCR
- In a plate file, in the **Run Method** tab, adjust the run method elements as needed.
For detailed instructions about editing the run method, see the primary user guides for the software.
- Click ... **(More Options)** ► **Filter Settings** to confirm or edit filter settings.

Confirm or edit the plate setup

For detailed instructions about plate setup, see the primary user guides for the software.

Add samples and assign to wells

For detailed instructions about plate setup, see the primary user guides for the software.

Note: Multiple targets can be assayed using standard curve analysis, but each target requires its own standard curve. To set up the standard curve using the **Standard Curve Wizard** see “Set up the standard curve” on page 11.

1. In the **Plate Setup** tab, add samples and assign to wells using the following options.
 - Import a plate setup file
 - Manually add samples to the **Samples** table
To assign a sample from the **Samples** table to the well, select a well in the plate layout, then select the checkbox associated with the sample in the **Samples** table.
 - Manually add samples to wells in the plate layout
The sample is added to the **Samples** table.
2. Confirm or edit sample information in the **Samples** table.

Column	Description
Name	Sample name
Color	Sample color
Type ^[1]	Standard curve analysis uses the following sample types. <ul style="list-style-type: none"> • Standard^[2] Note: You must enter the quantity for each standard sample in the Quantity column. • Unknown • Negative Control
Quantity (standard samples only)	Enter the quantity for the standard sample. Note: The quantity entered for a standard sample in the Samples table is used to populate the Quantity column for standard tasks in the Targets table (see “Add targets and assign to wells” on page 11).

^[1] For more information, see “Sample types for standard curve analysis” on page 19.

^[2] Each target requires its own standard curve.

3. Confirm or edit sample well assignments in the plate layout.

Add targets and assign to wells

For detailed instructions about plate setup, see the primary user guides for the software.

Note: Multiple targets can be assayed using standard curve analysis, but each target requires its own standard curve. To set up the standard curve using the **Standard Curve Wizard** see “Set up the standard curve” on page 11.

1. In the **Plate Setup** tab, add targets and assign to wells using the following options.

- Import an AIF file
- Import a plate setup file
- Manually add targets to the **Targets** table
- Manually add targets to wells in the plate layout
- Import TaqMan™ assay plate and card files

2. Confirm or edit target information in the **Target** table.

Column	Description
Name	Target name
Color	Target color
Task ^[1]	The software automatically assigns a task to the target in a well based on the sample type in that well. The following tasks are used for standard curve analysis. <ul style="list-style-type: none">• Standard• Unknown• Negative Control
Quantity (standard tasks only)	The quantity entered for a standard sample in the Samples table is used to populate the Quantity column for standard tasks in the Targets table.

^[1] For more information, see “Sample types for standard curve analysis” on page 19.

3. Confirm or edit target well assignments in the plate layout.

Set up the standard curve

Note:

- Multiple targets can be assayed using standard curve analysis, but each target requires its own standard curve.
- To import a standard curve from a different data file, see “(Optional) Edit standard curve analysis settings” on page 17.
- You can also set up the standard curve during sample setup (see “Add samples and assign to wells” on page 10).

1. In the **Plate Setup** tab, in the plate setup pane, click ⋮ **(More Options)** ▶ **Standard Curve Setup**. The **Standard Curve Wizard** opens.

2. In the **Standard Curve Wizard** pane, enter the sample name prefix.

3. Select the target for the standard curve.

Option	Instructions
Target previously defined	Select the target from the dropdown list.
Target not previously defined	<ol style="list-style-type: none"> 1. Type the target name, the press Enter. 2. Select a reporter from the dropdown list. 3. Select a quencher from the dropdown list.

4. Adjust the parameters for the dilution series if needed.

- **Number of points**—5 recommended
- **Number of replicates**—3 recommended
- **Starting Quantity**—The highest or lowest standard quantity, without units.

Note: The quantity must be greater than 0.

- **Serial Factor**

Note: The serial factor calculates quantities for all standard curve points.

- Starting quantity is the highest value—Select 1:10 to 1:2.
- Starting quantity is the lowest value—Select 2× to 10×.

5. Select an option to select the wells for the standard

- Select **Automatically**.
- Select **Manually**, then select wells using the displayed plate layout.

6. Select to arrange the standards in **Rows** or **Columns**.7. Click **Apply Standard Curve**, then click **Close** to return to the **Plate Setup** tab.

Edit reagent information

In the on-premise configuration, adding, editing, or deleting a reagent is a controlled function. The user role must have the permission of **Add/Edit/Delete Reagent**.

1. In the **Plate Setup** tab, in the **Targets/SNP Assays** table pane, click **Reagents**.2. In the **Reagents** table, perform one of the following actions.

- Click **+** (**Add**).
- Click **...** (**More Options**) ▶ **Export Reagents** to export reagents.
- Click **...** (**More Options**) ▶ **Import Reagents** to import reagents.
- Click **...** (**More Options**) ▶ **Scan Reagents** to scan reagents.

3. If you are manually adding reagents or editing reagents, enter the following information in the table.

- | | |
|-----------|-------------------|
| • Name | • Part Number |
| • Type | • Lot Number |
| • Barcode | • Expiration Date |

Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.

4. If you are scanning the reagent barcode, in the **Scan Reagent** dialog box, select or deselect the **Enable automatic parsing** checkbox.

5. If you are scanning the reagent barcode, when the **Scan Reagent** dialog box is displayed, use a barcode scanner to scan the reagent label.

Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.

The fields in the **Scan Reagent** dialog box are populated.

6. In the **Scan Reagent** dialog box, click **Add**.

7. (Optional) Click **✕ (Remove)** in the row of a reagent to delete it from the table.

Select a passive reference

In the on-premise configuration, editing the passive reference is a controlled function. The user role must have the permission of **Edit Passive Reference**.

The passive reference is set for the plate. The default passive reference is ROX™ dye.

1. In the upper-left corner of the **Plate Setup** tab, select a passive reference from the dropdown list.
2. (Optional) Save the plate file or data file.

Review and save the plate file

In Thermo Fisher™ Connect Platform, instruments are connected by the InstrumentConnect application.


The QuantStudio™ 6 Pro Real-Time PCR Instrument and the QuantStudio™ 7 Pro Real-Time PCR Instrument can connect to the software.

1. In the **Run Summary** tab, review the run method selections, then edit if needed.
2. Review the plate setup, then edit if needed.
3. (Optional) Click the barcode field, then scan the plate barcode.

4. (Optional) Select **Add to My Plates**.

This option allows you to create new plate files using the current plate file as a template.

5. Select an instrument from the list.

If the instrument does not appear on the list, click  **System ► Instruments** to add a new instrument.

6. Save the plate file.

Start the run on an instrument. For more information about starting a run, see the documentation for the instrument.



Perform standard curve analysis

Review results in the Amplification Plot

For detailed instructions about reviewing results in the **Amplification Plot**, see the primary user guides for the software.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.


1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. Review the amplification status for each well.
3. Review or edit threshold settings.
4. Review or edit baseline settings.

Select the Standard Curve Analysis Module

1. In an open data file, click **Actions** ▶ **Analysis Modules**.
2. In the **Analysis Modules** window, select **Standard Curve**, then click **Ok**.
The Standard Curve Analysis Module opens.

Click **Analyze**, then review the results in the **Standard Curve** tab.

Review results in the Standard Curve Plot

1. In the **Standard Curve** tab, in the plot pane, select a target from the **Targets** dropdown list.
2. In the plot pane, click , then select an option from the **Color By** dropdown list: **Target**, **Sample**, or **Task**.
The plot is displayed. The target, slope, R^2 value, Y-intercept, amplification efficiency, and error are displayed below the plot.
3. Confirm that the slope, R^2 value, amplification efficiency, and error meet the analysis criteria.
For more information, see “Standard Curve Plot overview” on page 20.
4. Visually check that all unknown sample C_q values fall in the standard curve range.

5. In the **Well Table**, confirm that the C_q values of all replicate samples meet the analysis criteria.
6. In the **Replicate Group Table**, review the quantity mean and quantity SD if needed.
If all of the C_q values for the combination of a sample and a target are undetermined, **Undetermined** is displayed in the **Quantity Mean** column and the **Quantity SD** column.
If some of the C_q values for the combination of a sample and a target are undetermined, the undetermined values are excluded. The values that are displayed in the **Quantity Mean** column and the **Quantity SD** column are based on the valid C_q values.

If the results do not meet the analysis criteria, troubleshoot using one of the following strategies:

- Omit wells, then reanalyze (see “Identify and omit outliers from standard curve analysis” on page 16).
- Repeat the plate run, adjusting plate file setup and analysis settings to improve results.

Identify and omit outliers from standard curve analysis

Outlier wells have C_q values that differ significantly from the average for the associated replicate wells. For C_q precision, consider omitting the outliers from analysis.

1. In the **Standard Curve** tab, in the plot pane, click on an outlier data point to highlight the well in the **Well Table** and in the **Plate Layout**.
Outlier wells can also be omitted in the **Quality Check** tab (see the primary user guides for the software).
2. Omit outliers.
 - In the **Well Table**, select **Omit** in the row of the outlier well.
 - In the **Plate Layout**, select **⋮ (More Options) ▶ Omit Wells**.
3. Click **Analyze** to reanalyze the data with any outliers removed.

(Optional) Review dye signal profile in the Multicomponent Plot

For more information about the **Multicomponent Plot**, see the primary user guides for the software.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Multicomponent Plot** from the dropdown list.
2. Review the signal profiles for the passive reference dye, reporter dye, and negative control wells.
3. Review the plot to confirm that there are no irregularities in the dye signals.

(Optional) Review signal profile in the Raw Data Plot

For detailed instructions about reviewing results in the **Raw Data Plot**, see the primary user guides for the software.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Raw Data Plot** from the dropdown list.
2. Click-drag the **Cycle Number** slider through all of the cycles, then confirm that each filter displays the characteristic signal increase.

(Optional) Edit standard curve analysis settings

Open the Standard Curve Analysis Module.

1. Click **Actions** ► **Standard Curve Analysis Setting**.
2. In the **General Setting** tab, edit the analysis settings if needed.

Standard Curve Analysis Option	Description
On Plate Standard Curves	Select to use the standard curve from the current data file. Click Export to export the standard curve.
External Standard Curves	Select to use a standard curve that was previously exported from another data file for analysis in the current data file. The two data files must be from the same instrument type, block type, and run method. <ul style="list-style-type: none"> • Click Import, navigate to the standard curve file, then click Open. • Click Delete to delete an imported standard curve. • Click Export to export the standard curve.

3. Click **Apply**.

The data is reanalyzed using the updated analysis settings.

Export the results

In the on-premise configuration, the location to save the file is defined in the export settings. For users with the permission of **Edit Export Destination**, the location can be selected for a file download. For users without the permission of **Edit Export Destination**, the location cannot be selected.

In the Thermo Fisher™ Connect Platform, the results are exported to the downloads folder of the computer. The file name and location cannot be selected.

For more information about setting up the export location, see the primary user guides for the software.

1. In the table pane, click the table associated with the results to export.
 - **Well Table**
 - **Replicate Group Table**
2. Click ... **(More Options)** ▶ **Export**.
In the Thermo Fisher™ Connect Platform, the file is downloaded.
3. *(Optional)* In the **Export CSV** dialog box, edit the file name in the **File Name** field.
The **File Name** field is populated with a default file name.
4. Click **Browse** to select a location to save the file.
The **Browse** button is not available in the on-premise configuration.
5. Click one of the following options.
 - Click **Download**, then select a location for the file download. This option is available for the on-premise configuration, for users who have the permission of **Edit Export Destination**.
 - Click **Save**. This option is available for the on-premise configuration, for users who do not have the permission of **Edit Export Destination**.
 - Click **Export**. This option is available in the desktop configuration.



About standard curve analysis

Overview of standard curve analysis

Standard curve analysis is used to determine absolute target quantity in samples.

For standard curve analysis, the software performs the following tasks.

1. The software measures amplification of the target in a standard dilution series and in test samples.
2. The software generates a standard curve using data from the standard dilution series.
3. The software uses the standard curve to interpolate the absolute quantity of the target in the test samples.

Sample types for standard curve analysis

Standard curve analysis includes the following sample types for each target of interest. Each unique target requires its own standard curve.

Sample type (Type column in Samples table)	Sample description	Automatic target task assignment ^[1] (Task column in Targets table)
Standard	<p>A sample that contains known quantities or known relative quantities of the target</p> <ul style="list-style-type: none">• For known quantities—quantify the target in the standard sample using an independent method• For known relative quantities—generate a relative dilution series of the target standards <p>Note: You must enter a quantity for each standard sample in the Samples table. Do not edit the quantity in the Targets table.</p>	Standard
Unknown	Test sample	Unknown
Negative Control	<p>Water or buffer</p> <p>No amplification of the target should occur in NTC wells.</p>	Negative control

^[1] The software automatically assigns a task to the target in a well based on the sample type in that well.

Standard Curve Plot overview

The **Standard Curve Plot** displays the standard curve for samples designated as standards. The software calculates the quantity of a target in an unknown sample using the standard curve.

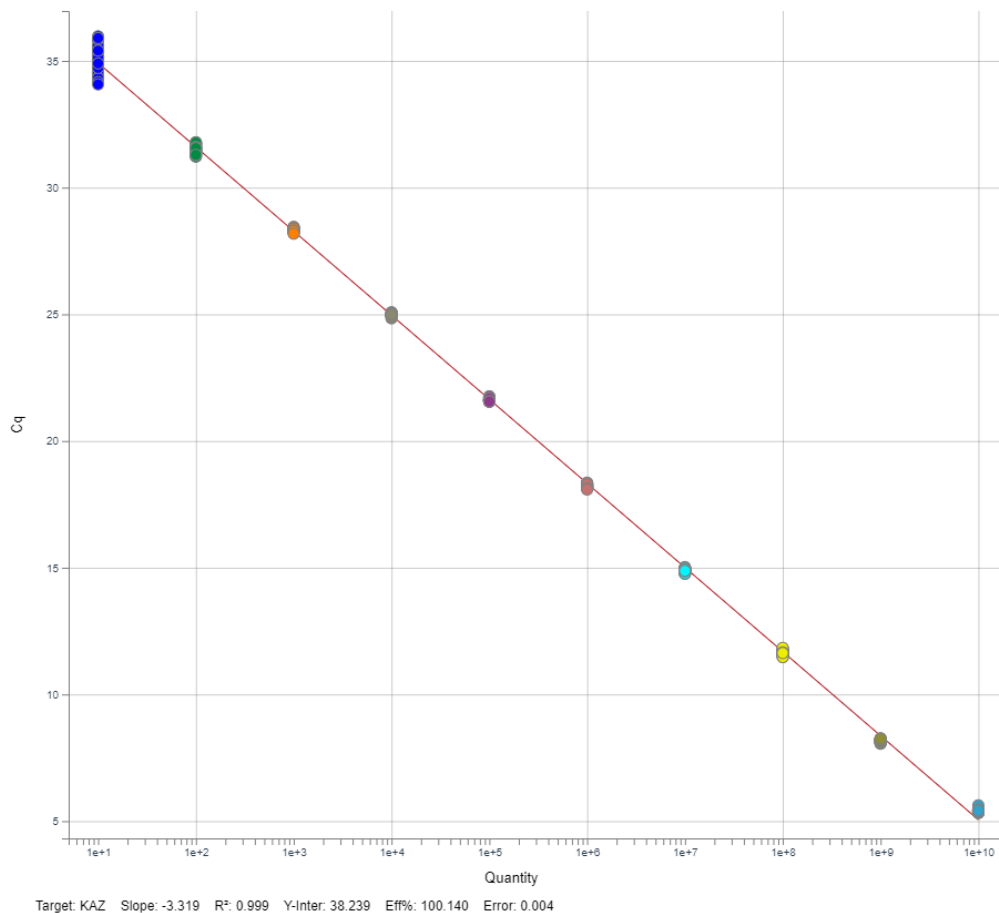


Figure 1 Example Standard Curve Plot

Table 1 Results or metrics to review in the Standard Curve Plot

Results or metrics	Description	Criteria for evaluation
Slope and amplification efficiency	The amplification efficiency is calculated using the slope of the regression line in the standard curve.	<p>A slope close to -3.3 indicates optimal, 100% PCR amplification efficiency.</p> <p>Factors that affect amplification efficiency:</p> <ul style="list-style-type: none"> • Improper design of the primer and probe • Range of standard quantities—For accurate and precise efficiency measurements, use a broad range of standard quantities, 5 to 6 logs (10^5- to 10^6-fold). • Number of standard replicates—For accurate efficiency measurements, include replicates to decrease the effects of pipetting inaccuracies. • PCR inhibitors—PCR inhibitors and contamination in the reaction can reduce amplification efficiency. • Other possible factors: <ul style="list-style-type: none"> – Component and properties of the reaction mix, such as salt content, DMSO, pH, etc. – Inaccurate sample or reagent pipetting – Improper analysis settings – Incorrect plate setup
R^2 value (correlation coefficient)	The R^2 value is a measure of the closeness of fit between the regression line and the individual C_q data points of the standard reactions.	<ul style="list-style-type: none"> • A value of 1.00 indicates a perfect fit between the regression line and the data points. • An R^2 value > 0.99 is desirable.
Error	<p>The standard error of the slope of the regression line in the standard curve.</p> <p>The error can be used to calculate a confidence interval (CI) for the slope and therefore the amplification efficiency.</p>	Acceptable value is determined by the analysis criteria.
C_q values	C_q is the PCR cycle number at which the fluorescence level meets the threshold.	<p>A C_q value > 8 and < 35 is desirable.</p> <ul style="list-style-type: none"> • C_q value < 8—There may be too much template in the reaction. • C_q value > 35—There may be a low amount of target in the reaction; for C_q values > 35, expect a higher standard deviation.



Documentation and support

Related documentation

Document	Publication number
<i>Diomni™ Design and Analysis (RUO) Software 3 (Desktop) User Guide</i>	MAN0030162
<i>Diomni™ Design and Analysis (RUO) 3 User Guide (Thermo Fisher™ Connect Platform)</i>	MAN0030163
<i>Diomni™ Design and Analysis (RUO) Software 3 (On-Premise) User Guide</i>	MAN1000091

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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