QuantiGene™ Plex Data Analysis Software

HELP

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About the QuantiGene™ Plex Data Analysis Software

The QuantiGene™ Plex Data Analysis Software enables you to analyze QuantiGene™ Plex gene expression assay results for up to 80 genes in a single well. The analysis features allow comparisons between individual samples within a plate, or groups of samples both within a plate and between different plates.

The QuantiGene™ Plex Data Analysis Software is compatible with all QuantiGene™ Plex assay kits. For more information, go to **https://www.thermofisher.com/us/en/home/life-science/gene-expression-analysis-genotyping/quantigene-rna-assays.html**.

Use the **Contents** navigation pane on the left to find a topic of interest, or enter a search term(s) in the **Search** field.

Features in this version

- Analyze data from 96-well and 384-well plates.
- Perform linearity of dilution calculations.
- View and export mean and individual sample values.
- Calculation of p-values during fold change analysis.
- Rank normalization genes for your analysis.
- Download a CHP file for use in Applied Biosystems™ Transcriptome Analysis Console (TAC).
- Group samples and analyze the fold change in up to five groups.
- Import and analyze Bio-Rad™ Bio-Plex files.
- Remove samples or targets with high fold change values from the data set.
- Proceed directly to analysis without assigning background wells.
- View QC values from the **Review** and **Result** screens.

Note: Click « **Return** at the top of each screen to return to previous screens in the workflow.

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Upload data files for analysis

IMPORTANT! Ensure that samples which are biological replicates are assigned different names before importing your data file. Samples with the same name are treated as technical replicates during analysis.

If you need to change the sample names, either edit the .csv file and assign unique names to biological replicates, or replay the plate with the xPONENT™ software using unique names for biological replicates.

In the **Dashboard** screen, upload CSV files for analysis using one of the following options.

Note: For the analysis to function properly, do not make any changes to the layout of the CSV files exported from your Luminex™ instrument.

| Option	Description
Import from **My Computer**	1. In the **Upload Files** pane, click 📁 **(My Computer)**. 2. Navigate to and select the data file, then click **Open**.
Drag and drop	Drag and drop the data file from your file browser to the **Upload Files** pane.

Set up your plate for analysis by assigning samples to different groups for comparison, assigning background wells and wells containing housekeeping genes for normalization, and removing any wells that you do not want to include in the analysis.

Assign well properties, then run the analysis

1. In the **Dashboard** screen, select a file from the **Recently Uploaded Files** pane. The **Plate Set Up** screen is displayed.

2. Click a well that you want to assign properties to.

   Note: All technical replicates of the sample are automatically highlighted when the well is selected.
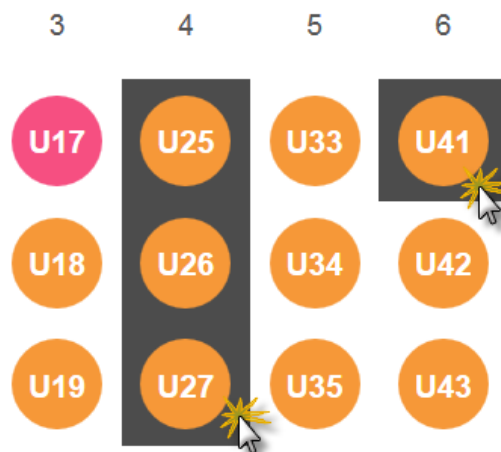
- **Click-drag** to select multiple contiguous wells
- **Ctrl+click** to select non-contiguous wells

3. Assign properties to the selected wells and edit samples (if needed).

Task	Action

Assign samples to a group	1. Click ✎ **(Edit) ▸ Edit Group**.
	2. Select **Unknown** from the dropdown list.
	3. Assign the sample(s) to a group from the dropdown list on the right.
	  • Select an existing group.
	  • Select **Modify Group** to create additional groups.
	4. Click **Save**.
	
	Note: A sample can only be assigned to one group.

Assign as background sample	**Note:** The software completes the analysis even if background samples are not assigned. 1. Click ✎ **(Edit) ▸ Edit Group**. 2. Select **Background** from the dropdown list. 3. Click **Save**.

(Optional) Remove a technical replicate or sample	Click ✎ **(Edit) ▸ Remove Replicate**, then click **+** to display the list of technical replicates for the sample.
	  • **Remove a replicate**—select the checkbox next to the technical replicate that you want to remove, then click **Save**.
	  • **Remove a sample**—select the checkbox next to all of the replicates for the sample, then click **Save**.

(Optional) Restore a technical replicate or sample	1. Click ☰ **▸ Restore Replicates**. 2. Select the checkbox next to each technical replicate, or click **Restore All** to select all of the replicates listed. 3. Click **Save**.

4. Select the normalization genes using one of the following options.

Option	Action
Select the normalization genes (without ranking)	1. Click ✂ **(Edit Normalization Genes)**, then click ⊕ next to the desired normalization gene. The selected gene is added to the **geNorm Ranking** pane. 2. Click **Save**. The selected normalization gene(s) is applied to all wells and all plates.
Rank, then select the normalization genes	1. Click ✂ **(Edit Normalization Genes)**, then click ⊕ next to each gene that you want to include in the ranking. The selected genes are added to the **geNorm Ranking** pane. **Note:** A minimum of four genes must be selected. 2. Click **geNorm Ranking** to calculate the stability measure (M) for each selected gene. **Note:** See **https://genomebiology.biomedcentral.com/ articles/10.1186/gb-2002-3-7-research0034** for details. 3. In the **geNorm Ranking** pane, select the checkbox next to the gene(s) that you want to use to normalize the data, then click **Save**. The selected normalization gene(s) is applied to all wells and all plates.

5. *(Optional)* To view calculated QC values, click 🔍 **(QC)** ▸ **View QC**, then select the QC parameter from the dropdown list to view the results.

   Note: Values that fail are shown in red.

   For more information on QC parameters, see "Review and edit QC parameters" on page 13.

6. *(Optional)* To set QC parameters, click 🔍 **(QC)** ▸ **QC Settings**, enter new values in the appropriate fields, then click **Apply**.

   For more information on setting QC parameters, see "Set QC parameters" on page 14.

7. Click 📄 **(Run)** to analyze the data.

Review results

After the QuantiGene™ Plex Data Analysis Software processes your experiment, the **Review** screen is displayed.

The left pane displays the median fluorescent intensity (MFI) of each well. The default toggle position of **Mean** shows the normalized mean MFI values for each biological replicate. A mean value with at least one failed QC parameter for a technical replicate is denoted with an asterisk (*). Selecting **Mean** causes subsequent analysis (**Sample** or **Group**) to be calculated using the mean MFI.

The **Individual Replicates** setting displays the individual MFI values for each technical replicate in the left pane. Selecting **Individual Replicates** causes subsequent analysis to be calculated using MFI.

Set the type of comparison to be performed using the other toggle in the right pane. The default toggle position of **Sample** calculates fold change values in relation to a selected reference sample.

The **Group** setting is used to compare groups assigned during plate set up to a selected reference group (for example, "treated" vs. "untreated").

Compare groups

Comparisons between groups can be performed within a single plate and between multiple plates.

1. In the **Review** screen, click the toggle to **Group**.

2. For **Reference Group**, select the group that you want to use as the standard for the fold change calculation.

3. From the **Group(s)** list, click ⊕ next to each group that you want to include for comparison.

4. Click **OK**.
   The **Result** screen is displayed.

Compare samples

Comparisons between samples can only be performed within a single plate.

1. In the **Review** screen, click the toggle to **Samples**.

2. For **Plates**, select the plate that contains the samples to be analyzed.

3. For **Reference Sample**, select the sample that you want to use as the standard for the fold change calculation.

4. From the **Samples** list, click ⊕ next to each sample that you want to include for comparison.

    Note: A maximum of five samples can be selected for comparison.

5. Click **OK**.
    The **Result** screen is displayed.

Select data to display

The **Result** screen displays the change in expression of selected targets with respect to the reference sample or reference group. If the value of a target in the reference sample or group is at or below the limit of detection, the target is automatically excluded from the data displayed in the graph. This ensures that fold change values reflect true differences in gene expression. In addition, p-values are calculated using Welch's test for group comparisons for which two or more sample values exist.

The software allows you to modify targets or samples that are displayed.
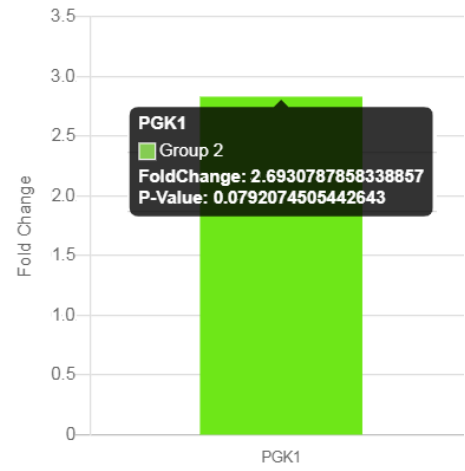
*   In the **Result** screen, select or deselect the appropriate checkbox to modify the targets and/or samples that are displayed in the bar graph.

- Hover over the fold change value in the table view or on the bar graph to see the p-value.

Review and edit QC parameters

QC parameters allow you to view and adjust the thresholds for data analysis. The following QC parameters can be modified:

- **Maximum Background**: The upper limit for which a value is considered to be within the background.

  This value can be set to zero, if you do not want to flag values with high background as failing in your data set.

- **Limit Of Detection**: The lower limit for which a value is considered to be detectable.

- **Technical Precision (%CV)**: The percent coefficient of variation for the value of replicate wells.

- **Saturation**: The upper threshold for the intensity of the signal used as a value.

  This value can be set to zero, if you do not want to flag values with high intensity as failing in your data set.

- **Minimum Bead Count**: The minimum number of beads allowed for inclusion of a data point.

Review QC values

Calculated QC values can be viewed from the **Plate Set Up** screen prior to running the analysis. Values that do not pass the set parameters can be viewed from the **Review** and **Result** screens after running the analysis.

To view calculated QC values, use one of the following options.

Option	Description
From the **Plate Set Up** screen (all values)	Click 🔍 **(QC)** ▸ **View QC**, then select the QC parameter from the dropdown list to view the results.
	Note: Values that fail are shown in red.
From the **Review** or **Result** screen (failed values only)	Click **Failed QC**, then select the QC parameter from the dropdown list to view the results.
	Note: Only values that fail are shown in the table.

Set QC parameters

QC parameter settings can be adjusted from the **Plate Set Up** or **Review** screen.

To set QC parameters, use one of the following options.

Option	Description
From the **Plate Set Up** screen	1. Click 🔍 **(QC)** ▸ **QC Settings**. **Note:** Alternatively, from the **Quality Control** screen, click ⚙ **Settings**. The **QC Settings** screen is displayed. 2. Enter the new values in the appropriate fields, then click **Apply**.
From the **Review** screen	1. Click **Failed QC**, then click **Reconfigure**. The **QC Settings** screen is displayed. 2. Enter the new values in the appropriate fields, then click **Apply**.

Calculate assay linearity

This function calculates the % recovery for a dilution series ("% recovery = observed value/expected value × 100%). Values that fall between 70–130 of the % recovery values indicate that the data are within the linear range of the assay.

1. Select all the wells that are part of the same dilution series.

2. Select 🔍 **(QC)** ▸ **Assay Linearity**

3. If necessary, click and drag rows so that they are arranged in descending order, with the first point in the dilution series at the top of the data table.

4. Enter the dilution factor used for the dilution series.

5. Click **OK**.

6. (*Optional*) Click ▤ **(Export)** to export results in Microsoft™ Excel™ or CSV format.

Data generated with the QuantiGene™ Plex Data Analysis Software can be exported to your PC for further examination.

Export normalized values

The **Review** screen shows the normalized values in the left pane.

1. Set the toggle in the right pane to **Mean** for mean values of samples, or **Individual replicates** for values for each technical replicate (recommended).

2. To download or export data, select from the following options.

   - Click ⬛ **(Export)** to export results in Microsoft™ Excel™ or CSV format.

     Note: A set of replicates with at least one failed QC parameter is denoted with an asterisk (*).

   - Click ⬇ **Download CHP** to download a CHP file for use in Applied Biosystems™ Transcriptome Analysis Console (TAC).

Export fold change values

The **Result** screen displays the change in expression of selected targets compared to the reference sample or reference group. Results display as a table with numeric values in the left pane, and as a bar graph in the right pane.

Note: A set of replicates with at least one failed QC parameter is denoted with an asterisk (*).

1. In the **Result** screen, select the preferred plot display for the data set.

   The fold change can be displayed as a linear value or as log values.
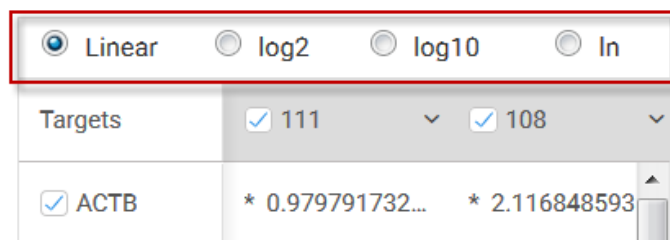
2. *(Optional)* Deselect the checkbox next to a sample or target that you want to remove from the data set.

Targets	○ Linear	○ log2	○ log10	○ ln
	☑ 104 ⌄	☑ 101 ⌄	☑ 10 ⌄	
☒ ACTB	0.6165971236...	0.9043736076...	0.3791477766...	
☑ CD3E	* 42.43605867...	* 30.17783370...	* 5.958362494...	
☑ CXCL10	21.768860838...	346.28532339...	9.5124734557...	

3. Click 🖹 **(Export)** to download data in PNG (bar graph), Microsoft™ Excel™, or CSV format.

Note: A set of replicates with at least one failed QC parameter is denoted with an asterisk (*) in exported Microsoft™ Excel™ or CSV files.

Documentation and support

Customer and technical support

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- Worldwide contact telephone numbers
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  - Product FAQs
  - Software, patches, and updates
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- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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