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About ProteinAssist™ Software

ProteinAssist™ Software performs relative quantification calculations for your TaqMan® Protein Assay CT data. TaqMan Protein Assays enable detection and relative quantification of protein targets using an adapted form of PLA™, a proximity ligation assay technology, in combination with real-time PCR. Information about TaqMan Protein Assays and key differences in data analysis of TaqMan Protein Assays and traditional real-time PCR is provided in Appendix A on page 38. The algorithm used to calculate fold change (relative quantification) is described in Appendix B on page 40.

About studies and experiment files

In the ProteinAssist Software, data is managed as a study.
- A study is a collection of TaqMan Protein Assay experiment files.
- An experiment file contains TaqMan Protein Assay data from a single reaction plate. The reaction plate must be from one of the Applied Biosystems real-time PCR systems listed under “Compatible real-time PCR systems.”

A study allows you to analyze data from multiple plates, with an option of using one reference sample for all the plates for that assay.

Experiment file criteria

Each experiment file must have a unique file name.

ProteinAssist™ Software workflow

Table 1. Workflow steps and key software tasks

<table>
<thead>
<tr>
<th>Workflow step</th>
<th>Key tasks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1: Set up a study.</td>
<td>• Set up reference sample use: per plate or per study.</td>
</tr>
<tr>
<td></td>
<td>• [Optional] Set up input quantity units for display.</td>
</tr>
<tr>
<td></td>
<td>• [Optional] Set up custom fields.</td>
</tr>
<tr>
<td>Workflow step</td>
<td>Key tasks</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
</tr>
</tbody>
</table>
| **Step 2: Set up the experiment files within a study.** | • Import Ct data from real-time PCR instrument plate files.  
• *(Optional)* Incorporate multiple plates into a single study.  
• Assign required well attributes:  
  – Input quantity.  
  – Task: NPC (no protein control), reference, or unknown.  
  – Sample name.  
  – Assay name.  
  **Note:** A combination of these methods can be used to easily assign well attributes:  
  – Well Editor  
  – Samples lists  
  – Assays lists  
  – Well Explorer  
  – Plate templates  
• *(Optional)* Assign other attributes to the wells (for example, group, treatment, time of treatment, or custom attributes). |
| **Step 3. Analyze a study and view the analysis results.** | • View and ΔCt and Ct values in both graphical and tabular format (Linear Range).  
  – Examine the data for outliers.  
  – Verify or modify the linear range automatically calculated by the software.  
• View fold change values as a bar graph with nested grouping (Fold Change).  
• View fold change values as a heat map (Heat Map; this feature is useful when viewing results from many samples and assays). |
| *(optional)* **Step 4. Export study data** | • Export study data in a spreadsheet-compatible format.  
• Export study data in a ProteinAssist™ Software-compatible format. |
Compatible real-time PCR systems

Table 2. Real-time PCR systems and software compatibility

<table>
<thead>
<tr>
<th>Real-time PCR system (Fast system recommended)</th>
<th>Plate file extension</th>
<th>System software</th>
</tr>
</thead>
<tbody>
<tr>
<td>7500 Fast system</td>
<td>*.eds</td>
<td>7500 Software v2.02</td>
</tr>
<tr>
<td></td>
<td>*.csv</td>
<td>SDS Software v1.4</td>
</tr>
<tr>
<td>7900HT/7900HT Fast system</td>
<td>*.txt</td>
<td>SDS Software v2.3 Patch A, B, C</td>
</tr>
<tr>
<td>Standard 96-Well Block Module</td>
<td></td>
<td></td>
</tr>
<tr>
<td>384-Well Block Module</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast 96-Well Block Module</td>
<td></td>
<td></td>
</tr>
<tr>
<td>StepOnePlus™ system</td>
<td>*.eds, *.csv, *.txt</td>
<td>StepOne™ Software v2.1</td>
</tr>
<tr>
<td>ViiA™ 7</td>
<td>*.txt, *.csv</td>
<td>ViiA™ 7 Software V 1.0</td>
</tr>
</tbody>
</table>

Key toolbars and software conventions

Home screen toolbar
When you first open the software, the home screen will display. Use the home screen toolbar to manage studies (workflow steps 1 and 4).
Analysis toolbar

Use this toolbar in all steps of the software workflow. The analysis toolbar remains unchanged in all the Setup, Analysis, and Export screens.

Note: It is prudent to save your studies regularly, because there is no autosave feature in ProteinAssist software. In the analysis toolbar, select Save or Save as.

Experiments toolbar

Use the Experiments toolbar to set up experiment files for analysis (workflow step2). The Experiments toolbar is located on the Setup > Experiment Files screen.
Field error icon

The field error icon indicates that an experiment file is not set up properly. Mouse-over the icon for information on how to correct the error.

### Manage the software

#### System requirements

<table>
<thead>
<tr>
<th>Minimum Hardware Configuration</th>
<th>Recommended Hardware Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intel CPU 2.4 GHz</td>
<td>Intel CPU 3.0 GHz</td>
</tr>
<tr>
<td>Available hard disk space: 1 GB</td>
<td>Available hard disk space: 20 GB</td>
</tr>
<tr>
<td>Memory: 1 GB</td>
<td>Memory: 2 GB</td>
</tr>
</tbody>
</table>

**Operating System:** Windows® XP 32-bit (recommended Service Pack 3)

#### Install the software

2. Follow the prompts for downloading the ProteinAssist™ Software.
   - **Note:** If you are re-installing ProteinAssist Software, we recommend that you first transfer out your studies. See “Transfer out a study.”
3. Navigate to the folder containing the downloaded installer, and double-click `setup.exe` to start the installation.
4. Follow the prompts of the InstallShield Wizard to install the ProteinAssist software in the desired location.
   - **Note:** If you have a previously installed version of ProteinAssist Software, you will need to first uninstall the software.
Uninstall the software

If you are re-installing the pre-release ProteinAssist Software, the existing software must first be uninstalled. You may be prompted with the following messages.

- The uninstall process will prompt you to back up your data within the application workspace; see the messages displayed below.
- To save your study outside the application workspace, transfer out a study before starting the uninstall process. See page 34.

**IMPORTANT:** You must transfer the study to a folder outside of C:\Applied Biosystems\Protein Assist Software; otherwise it will be overwritten when the software is reinstalled.

<table>
<thead>
<tr>
<th>Message</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Question" /></td>
<td>Select Yes; you must uninstall the existing version of the software to proceed.</td>
</tr>
<tr>
<td><img src="image2.png" alt="ProteinAssist(TM) Software - InstallShield Wizard" /></td>
<td>Select OK after closing ProteinAssist™ Software and other applications on your computer.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Backup Data" /></td>
<td>Select Yes to back up your data within the software workspace. (Files are transferred to C:\Applied Biosystems\Protein Assist Software Backup).</td>
</tr>
</tbody>
</table>
5. Once the uninstall is complete, run the installation program again to re-install the software. Double-click setup.exe again.

6. To restore your data:
   - **If you backed up your data within the software workspace**: follow the prompts to restore your data during the re-installation.
   - **If you transferred out your study**: in the Home screen, select **Transfer in Study**, navigate to the folder where your backup study files reside (*.las), and select the study file for import.

### Set preferences

In the global menu bar, select **Tools > Preferences**.
Set default home location

On the General tab, select the location to store transferred-out studies and exported files:

- **User-defined location:** Select Browse to navigate to your selected folder.
- **Last-accessed location:** The folder you last visited, in or out of the application.

Set study preferences

On the Study Preferences tab, set the default settings for all studies.

- **Study Properties:** Enter the units of the input quantities for the samples. These units will display in the data plots and at other points in the software.
- **Reference Use:** Select the reference sample use for data analysis.
  - **Per Plate:** Each assay must have a reference sample assigned in the same plate.
  - **Per Study:** Each assay must have a reference sample assigned, but it can be in a different plate within the same study.
- **Decimal Delimiter:** Numerical values in exported .txt files can either have a period (.) or a comma (,) as the decimal delimiter.
- **Show Wells Fields:** Select the Well Fields to be displayed in the Plate Layout screen and the well editor. Note that Sample, Assay, Task and Input Quantity are required fields for analysis.
- **Analysis Settings:** Set the default settings for Outlier Detection, Linear Range Detection and Threshold Setting.
  - **Outlier Detection:** Outlier wells will be flagged in the Plate Layout and the Well Table based on user-specified Sensitivity (standard deviations from the average of replicate Ct values).
  - **Linear Range Detection:** The regression linear range of the dilution curve for each sample is automatically computed when you click on Analysis in the Analysis toolbar. The linear range algorithm evaluates whether or not
to include a data point by determining how far a given value deviates from the regression line. The sensitivity of the linear range algorithm is the degree to which the average ΔCt values of replicate groups must be collinear. Adjust the sensitivity as follows.

- **High sensitivity:** A replicate group is considered part of the linear range only if its average ΔCt value falls very close to the regression line of an initial set of two or three replicate groups chosen by the algorithm.

- **Low sensitivity:** A replicate group can be considered part of the linear range even though its average ΔCt value deviates significantly from the regression line of an initial set of two or three replicate groups chosen by the algorithm.

- **Quantification Threshold:** Samples with all ΔCt values below the Quantification Threshold will not be analyzed, and they will be designated “Undetermined.” The default value of the Quantification Threshold is 2.0.
Set up and manage a study

When you open the ProteinAssist Software, the Home screen is displayed. In the Home screen, you launch existing studies, or create new studies.

Create a study

1. In the toolbar on the Home screen, select Create Study.

The following steps refer to the screenshot on the next page.

2. The Properties screen automatically opens.
   - To edit an existing study: in the Study Workflow pane, select Setup > Properties to open the Properties screen.

3. Enter a Study Name.

4. (Optional) Enter the units of the input quantities for the samples. These units will display in the data plots and at other points in the software.

5. Select the reference sample use for data analysis.
   - Per Plate: Each assay must have a reference sample assigned in the same plate.
   - Per Study: Each assay must have a reference sample assigned, but it can be in a different plate within the same study.

6. (Optional) Enter custom field names. These fields can hold additional data relevant to your experiment.
   - For example, you could assign a custom field name of “Cell Line.” You can then enter the name of the cell line for that sample when you set up the plates.
   - Note: Custom field names cannot be empty. If you do not assign custom field names, leave the default values in the fields.

7. (Optional) Enter a description of the study.

8. (Optional) In the Comments field, enter comments for the study, and select Add. The software records the comments and the date/time you added the comments. The Comments field allows you to enter detailed information about the study (for example, observations about the data, reasons why you made specific decisions, and so on).
   - IMPORTANT: After you select Add, the comment is permanently recorded in the study (that is, the comment cannot be modified or removed).

9. In the toolbar on the Properties screen, select Save to save the study.
   The study is saved in the application workspace. It can be accessed through the Home screen.
Transfer in a study

1. In the toolbar on the Home screen, select **Transfer in Study**.
2. Navigate to the location of your saved study file (*.las).
3. Select the file, then click **Open**. The Properties screen will be displayed.

Example study

An example study file, Example Study v1.0.las, is installed with the ProteinAssist Software. Transfer in this study as described above to help learn about software features. It is located at C:\Applied Biosystems\ProteinAssist Software\User Data\examples

- The study includes 3 experiment files, with 4 samples, and 3 assays.
- Input quantity unit is set to “cells/well.”
- Reference use is set to “Per Plate.”

Transfer out a study

Studies are saved within the ProteinAssist Software workspace. To generate a study file that can be saved as an independent file (to email, for example), the study must first be transferred out. See page 34.
Add experiment files to a study

ProteinAssist Software is compatible with experiment files containing Ct values as described in Table 2 on page 7. For some instruments, the experiment file may include sample name, input quantity, or assay name. Only .eds, .txt or .csv files can be imported.

1. In the Study Workflow pane, select **Setup > Experiment Files** to open the Experiment Files screen.

2. In the Experiment Files toolbar, select **Import** and navigate to the file(s) to be imported. You can select and import multiple Experiment Files at the same time by using the Ctrl key.

Example experiment files (*.txt) are located in C:\Applied Biosystems\Protein Assist Software\User Data\examples.

**Note:** Each Experiment File must have a unique name.

3. Click on file name(s) and select **Import**.

**IMPORTANT:** Changing the content of .txt or .csv files may prevent importing the files, or it may result in incorrect analysis in ProteinAssist Software.

Set up experiment files for analysis

ProteinAssist™ Software provides a number of flexible features for setting up your experiment files, described in the following section.

**Note:** Save your studies regularly, because there is no autosave feature in ProteinAssist software. In the analysis toolbar, select **Save** or **Save as**.
Analysis rules

All experiment files within a study must be set up with specific parameters that follow the analysis rules in order for analysis to proceed. These analysis rules can also be accessed by clicking the icon in the Experiments toolbar:

1. The study must have wells to analyze. It does not support cases where all wells are omitted.
2. All included wells must have Sample, Assay, Task and Input Quantity specified.
3. Each assay-sample combination must be unique across the study.
4. Every assay-sample combination should have at least two input quantities (not including the NPC).
5. Each assay-sample combination must be assigned with only one task, either "Unknown," "Reference," or "NPC."
6. Every experiment file must have at least one NPC well for each assay assigned in the experiment.
7. Every assay must have at least one unknown sample.
8. If Reference Use is Per Study: Every assay must have one reference sample, but it can be in a different plate/experiment file within the same study.
9. If Reference Use is Per Plate: Every assay must have one reference sample in the same plate/experiment file.

Set up an experiment file using the Well Editor

1. Select Setup > Experiment Files, and select an experiment file from the list of imported files.
2. In the Plate Layout tab, select a single well, or click and drag to select multiple wells.
   
   **Note:** When you click and drag multiple wells, ensure that the cross-hair icon is **not** displayed. If you select wells using the cross-hair, you will change the input quantity of the selected wells, as described in “Set up a dilution series,” following.
3. Right-click the mouse to access the well editor and select **Edit Well(s)**. You can also access the well editor for a single well by selecting the well and either double-left-clicking the mouse or hitting the **Enter** key.

4. Enter the required fields: **Sample, Input Quantity, Task and Assay**. See Table 3.

5. (Optional) Assign other attributes to samples for sorting and display purposes.
   a. Select **Show in Wells** in the Plate Layout toolbar, and check one or more of the following fields: **Group, Treatment, Time, Custom 1, Custom 2, Custom 3**. The selected field(s) will now appear in each well and in the edit well window.
   
   **Note:** Custom 1, Custom 2 and Custom 3 can be renamed in **Setup > Properties**.

   b. Right-click the mouse over selected wells to enter the field values as in step 3.

### Table 3. Fields required for analysis

<table>
<thead>
<tr>
<th>Field</th>
<th>Action</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Type in a new sample name. -or- Select a sample name from the drop-down menu.</td>
<td>Sample names will be imported from the Experiment Files if sample names were assigned within the instrument software. Sample names will automatically appear in the Sample list to the left of the Plate Layout pane after typing in a new sample name or upon import of an Experiment File with pre-assigned sample names.</td>
</tr>
<tr>
<td>Input Quantity</td>
<td>Type in a numerical input quantity for the sample.</td>
<td>Input values will be imported if quantity values were assigned within the instrument software. At least two different input quantities are required per sample/assay combination.</td>
</tr>
</tbody>
</table>
### Field | Action | Notes
--- | --- | ---
Task | Select Reference, Unknown or NPC. | Reference: A reference sample is required for each assay, and it is assigned per plate or per study [see Properties]. Unknown: Test sample. NPC: No Protein Control. There must be at least one NPC per assay per plate. Wells with NPC are automatically assigned Input Quantity of zero.
Assay | Type in a new assay name - or - Select an assay from the drop down menu. | Target names will be imported as Assays from the Experiment Files if target names were assigned within the instrument software. Assay names will automatically appear in the Assay list to the left of the Plate Layout pane after typing in a new assay name or upon import of an Experiment File with pre-assigned target names. The ProteinAssist™ Software has 6 assays pre-loaded for your convenience: hCSTB, hICAM1, hLIN28, hNANOG, hOCT3/4 and hSOX2

### Set up a dilution series

A dilution series can be easily set up after Input Quantity has been assigned to the well(s). This feature is similar to a click and drag autofill function in spreadsheet software.

1. Enter the desired dilution factor in the Plate Layout toolbar.
2. Select one or more wells.
3. Hover the mouse over the lower right corner of the selected well(s) until a crosshair appears. Drag the crosshair to include all the wells of the dilution series. The Input Quantity from the first well, row, or column selected will be reduced by the dilution factor in each succeeding well.

**Note:** All attributes, other than Input Quantity, will be copied into the included wells.
Copy or edit well attributes

Copy and paste all well attributes
All of the attributes from one or more wells can be copied and pasted into other wells. This feature is similar to copy and paste functions in spreadsheet software.

1. Select wells to be copied one of the following ways:
   • Select an entire row by clicking on A, B, C….H in the Plate Layout.
   • Select an entire column by clicking in 1,2,3…..12 in the Plate Layout.
   • Select one or more wells by left-clicking and dragging the mouse over the desired wells.
   • Select the entire plate by clicking on the upper left corner in the Plate Layout.
2. Right-click to access to the well editor and select Copy.
3. Select other wells for pasting the well attributes into and select Paste.

Assign sample or assay names using the Samples or Assays lists
The Samples and Assays lists are located to the left of the Plate Layout tab.

1. Select one or more wells.
2. Check the box next to the sample or assay name in the appropriate list.

Assign well attributes using the Well Explorer
For an individual well, all well attributes except well location, task, and Ct value can be changed in the Well Explorer.

1. Select a well. The assigned attributes are displayed in the Well Explorer.
2. Click the value for the attribute to be changed. If an attribute if editable, the value will be highlighted.
3. Type in the value for the field. New values for sample and assay names are updated in the Samples and Assays lists.
**Edit well attributes using Excel**

1. Select one or more wells and right-click to access the well menu. Select **Copy**.
2. Open up a blank Excel worksheet and use the Paste function to copy the well attributes into the worksheet.
3. After making the desired edits, copy all rows and columns with data or annotation, including column headers.
4. Right-click on the top-left well of the selected group in the Plate Layout to paste the edits for all the selected wells.

---

**Re-use well attributes using plate templates**

**Generate Plate Template**

After the well attributes have been assigned, you can save the plate configuration as a plate template.

It is possible to generate a template without one or more of the required attributes (such as Sample).

1. Select **Generate Plate Template** in the Experiments toolbar and name the template.
2. Click on **Generate** to save the new template.
Load Plate Template

To use a previously generated Plate Template:

1. Select an Experiment File from the list and select **Load Plate Template** in the Experiments toolbar.
2. Select a plate template and click **Load**.

Omit or include wells from analysis

- **Omit wells**: Select one or more wells and click on the Omit icon in the Plate Layout toolbar.
- **Include omitted wells**: Select one or more of the omitted wells and clicking on the same Omit icon.

**Note**: A well with no CT, CT ≥ 40, or CT ≤ 0 is automatically omitted by the software and cannot be included.

Show in Wells: data, sample and assay color

- Select data to show or hide in the wells by clicking on the **Show in Wells** icon in the Plate Layout toolbar.
- Select to differentiate either each sample or each assay by color or choose not to have any color-coded display in the wells.
Well Table tab: view data and attributes in table format

Select the Well Table tab to view all the data and attributes associated with the Experiment File.

- **Sort the data**: click on the column header of interest.
- **Perform multiple sorts**: click on the first column header of interest, then control-click on the next column header of interest. Continue the sort by control-clicking on each column header of interest.
- **Hide or reveal columns**: select Show in Table in the Well Table tab toolbar and select or deselect fields in the drop-down menu.
- **Re-order the columns**: click on a column header and drag it to a new location in the table.
- **Export the table**: select Export in the Experiments toolbar.
Manage assays

In the Study Workflow pane, select Assays to open the Assays screen.

- The six commercially available assays from Applied Biosystems have been included here for your convenience.
- **To add new assays**, click on `Add` and type in the new assay name.
- If desired, **change the color** associated with each assay that appears in the wells of the Plate Layout using the drop-down menu.
- **Remove assays** that are not currently in use in the study by selecting `Remove`.

![Assays Table]

Manage samples

In the Study Workflow pane, select Samples to open the Samples screen.

- **To add new samples**: click on `Add` and type in the new sample name.
- If desired, **change the color** associated with each sample that appears in the wells of the Plate Layout and in the Linear Range plots within Analysis: use the drop-down menu.
- **Add, delete or edit sample attributes** associated with the Group, Treatment, Time and Custom fields: double-click in the field of interest for that sample and edit its value.
- **Remove samples** that are not currently in use in the study: click on `Remove`.

![Samples Table]
Analyze a study

After all the mandatory well attributes have been assigned (sample name, assay name, task and input quantity) to each experiment file in the study, select Analysis in the Study Workflow pane.

Note: If the experiment files have not been set up correctly, an error message will appear when you select Analysis. See “Troubleshooting a failed analysis” on page 37. Field error icons are cleared only after a study is re-analyzed after correcting setup errors.

View the ΔCₜ plot

Select Analysis > Linear Range > ΔCₜ Plot tab. Select an assay from the drop down Assay menu in the ΔCₜ plot toolbar.

ΔCₜ and average ΔCₜ

The ΔCₜ and average ΔCₜ values for each sample-assay combination are plotted against the input quantities. The ΔCₜ and average ΔCₜ values are plotted as solid circles and triangles, respectively, on the plot. The NPC ΔCₜ and average NPC ΔCₜ values are plotted as open black circles and solid colored triangles, respectively.

- ΔCₜ = average NPC Cₜ – sample Cₜ
- average ΔCₜ = average NPC Cₜ – average sample Cₜ
- NPC ΔCₜ = average NPC Cₜ – NPC Cₜ
- average NPC ΔCₜ = average NPC Cₜ – average NPC Cₜ (=0)

Quantification Threshold

The Quantification Threshold is shown on the ΔCₜ plot as a horizontal dashed line. The Quantification Threshold:

- Is set above the background noise (default value: 2.0). Samples with all ΔCₜ values below the Quantification Threshold will not be analyzed; they will be designated as “Undetermined”.
- Is a factor used in the calculation for the fold change between the Unknown and Reference samples.
- Can be changed by clicking and dragging the horizontal dashed line up or down or by typing in the threshold value in the ΔCₜ Plot toolbar. Click on Analyze in the toolbar to recalculate the plot with the new Quantification Threshold.
Automatic Linear Range

- The software automatically determines a set of data for each sample that follows a linear relationship between log(input quantity) and ΔC\textsubscript{T}. This set of data is used in the relative quantification calculation.
- The gray zone (see screenshot following) encompasses the upper and lower boundaries of the data set, and is called the linear range.
- For each sample, a dotted line shows the regression line for that set of data.
- When you open the ΔC\textsubscript{T} plot the first time for each study, all the samples and regression lines are displayed. The lowest and highest values of the linear ranges for all the samples are also displayed.
- To display the linear range for a sample-assay combination: click on the sample-assay combination in the sample table below the plot.
Set the Linear Range manually

To manually adjust the range used to calculate the linear range of one or more samples:

1. Click on the sample curve(s) in the ΔCt plot or the sample row(s) in the table below the ΔCt plot.
2. Click and drag the left and right bars to adjust the upper and lower boundaries of the linear range.
3. Click on Analyze in the toolbar for reanalysis.
Omit wells
Outliers are automatically detected by the software and the individual wells are flagged on the ΔC\text{r} plot as open triangles.
1. Zoom in to facilitate selecting outlier data points by clicking on **Zoom In** and using the mouse to encircle the region to enlarge.
2. Omit the data point by clicking on the open triangle and selecting **Omit**. Omitted wells can be included by selecting **Include**.
3. After data omission or inclusion, the software automatically re-analyzes the data.

Change the data and tooltips displayed in the ΔC\text{r} plot
You can hide or include ΔC\text{r} and Avg ΔC\text{r} data points, Avg ΔC\text{r} curves, regression lines, omitted wells and the Quantification Threshold Line on the ΔC\text{r} plot in the **Show** menu in the ΔC\text{r} plot toolbar.

You can hide or include Sample, Task, Input Quantity, ΔC\text{r}, Experiment and Location of well in the tooltip in the **Tooltips** menu in the ΔC\text{r} plot toolbar.
Change the analysis settings

1. Change default Outlier Detection and Linear Range Detection settings by clicking on **Analysis Settings** in the toolbar (refer to Study Preferences on page 12 for descriptions).

2. Click on **OK**. The software automatically performs a re-analysis with the new setting(s).

![Analysis Settings]

View the sample table

The sample table located below the ΔCₜ plot contains all the data and attributes associated with each sample.

- The data in each column can be sorted by clicking on the column header.
- Columns can be resized, hidden or revealed by selecting **Show in Table** in the toolbar and selecting or deselecting the different fields.
- Checking/unchecking the box in the Show column will display/remove the sample ΔCₜ curve from the ΔCₜ plot.

<table>
<thead>
<tr>
<th>Column heading</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold Change</td>
<td>The fold change is automatically calculated for each sample with task assigned as Unknown. The fold change for samples assigned as Reference is N/A. Samples with all ΔCₜ values below the Quantification Threshold are assigned &quot;Undetermined.&quot;</td>
</tr>
<tr>
<td>Slope, Intercept, R²</td>
<td>Values for the slope, intercept and R² of the regression line for each sample.</td>
</tr>
<tr>
<td>Min and Max Input</td>
<td>The minimum and maximum input quantity values of the linear range.</td>
</tr>
</tbody>
</table>
**View the C\text{\textdegree} plot**

The C\text{\textdegree} plot is useful for viewing the plotted NPC values for troubleshooting purposes.

**C\text{\textdegree} and average C\text{\textdegree}**

The C\text{\textdegree} and average C\text{\textdegree} values for each sample/assay combination are plotted against the input quantities. The C\text{\textdegree} and average C\text{\textdegree} values are plotted as solid circles and triangles, respectively, on the plot. The NPC C\text{\textdegree} and average NPC C\text{\textdegree} values are plotted as open black circles and solid colored triangles, respectively.

**Omit wells and change display and tooltips in the C\text{\textdegree} plot**

The tools for omitting wells and changing the well data display and tooltips are the same as in “View the ΔC\text{\textdegree} plot” on page 26.
View the fold change results as a bar graph

Select **Analysis > Fold Change** in the Study Workflow.

- The fold change results can be changed from Log₂ (default) to Log₁₀ or linear formats using the drop-down **Type** menu in the Fold Change toolbar.
- The results are automatically grouped by assay. You can then choose to group by another factor using the drop down **Group by** menu in the toolbar. If **Group by > Sample** is selected, the sample name will appear on the X-axis.
- The data within the chart can also be sorted by different factors using the drop-down **Sort by** menu in the toolbar.
- Use the **Show** drop-down menu in the toolbar to display or hide the sample information (sample name and any assigned attributes). Note that the sample information will be displayed on the chart next to the bars.
**View the fold change results as a heat map**

The heat map display is useful when there are many assays and samples. Select Analysis > Heat Map in the Study Workflow.

1. The fold change values in the Heat Map can be viewed as Log₂ (default) to Log₁₀ by selecting the appropriate radio button in the Heat Map toolbar.
2. The data can be sorted by clicking on Specify Sort in the toolbar and selecting one or more sample attributes.

**Save and export study data**

<table>
<thead>
<tr>
<th>Task</th>
<th>File type</th>
<th>Menu commands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Save the plate layout view as an image file</td>
<td>*.jpg</td>
<td>Setup&gt;Experiment Files&gt;Plate Layout tab&gt;Save as Image</td>
</tr>
<tr>
<td>Save the ΔCₚ plot as an image file</td>
<td>*.pdf, *.png, *.jpg</td>
<td>Analysis&gt;Linear Range&gt;ΔCₚ Plot tab&gt;Save as Image</td>
</tr>
<tr>
<td>Save the Cₚ plot as an image file</td>
<td>*.pdf, *.png, *.jpg</td>
<td>Analysis&gt;Linear Range&gt;Cₚ Plot tab&gt;Save as Image</td>
</tr>
<tr>
<td>Save the Fold Change bar graph as an image file</td>
<td>*.pdf, *.png, *.jpg</td>
<td>Analysis&gt;Fold Change&gt;Save as Image</td>
</tr>
</tbody>
</table>
## Transfer out a study

The **Transfer out Study** function allows you to move a study for backup or sharing purposes.

The software stores studies as internal files in the application workspace. These internal files are not accessible to users. The only way to access a study created in another ProteinAssist Software application is to transfer it in. (This process is described in Transfer in a study on page 15.) Conversely, the only way another user can access a study created in your ProteinAssist Software application is for you to transfer the study out.

1. In the Home screen, select the study of interest, then select **Transfer out Study**.
2. Browse to a save location, enter a name for the study file (*.las), then select **Save**.
   If an existing study file (*.las) has the same name as the study you are transferring out, you will be asked if you want to overwrite the existing file.
   **IMPORTANT:** If you overwrite the existing study file, all the information in the existing study file will be lost.

The complementary function, **Transfer in Study**, allows you to import a study file that has been transferred out from the software. See page 15.

## Export study data

Export study data to a file compatible with downstream software such as Microsoft® Excel® software. Each study is organized into two types of data for export.

- Well properties and data: Properties and data that can be individually assigned to a well, including \( C_T \) and \( \Delta C_T \) values, and average values, standard deviation, and confidence intervals for replicates.
Fold change results: Fold change data for each sample, as well as relevant sample properties.

Export to a single file
In this option, the well properties and data and the fold change results are exported to a single file.

1. Set the location of the exported file. Accept the default location, or select browse to navigate to the desired save location.
2. Set the name and format of the exported file.
   a. Modify the default file name, if necessary. The default file name is:
      
      StudyName_yyyymmdd_hhmmss_Tables
      
      where
      - StudyName is the name of the current study
      - yyyymmdd and hhmmss are the current date and time
   b. Select the file type from the pulldown menu. Both file types are compatible with a spreadsheet application such as Microsoft® Excel® Software.
      - *.csv: comma-separated value-file
3. Select the data to be exported.
   a. Under the Properties & Data tab, check the properties and data to be exported.
   b. Under the Fold Change Results tab, check the properties and data to be exported.
4. Select/deselect open after exporting, then select export.
   If open after exporting has been selected, the software will open a *.txt file in a text editing program, and it will attempt to open the *.csv file in your default spreadsheet software.
Export to separate files

In this option, the well properties and data are exported to one file, and the fold change results are exported to a separate file. For each file export, do the following.

1. Set the location of the exported file. Accept the default location, or select **Browse** to navigate to the desired save location.
2. Set the name and format of the exported file.
   a. Modify the default file name, if necessary. The default file names are:
      - StudyName_yyyymmdd_hhmmss_Plate Setup for well properties and data
      - StudyName_yyyymmdd_hhmmss_Analysis Results for fold change results where
        - StudyName is the name of the current study
        - yyyymmdd and hhmmss are the current date and time
   b. Select the file type from the pulldown menu. Both file types are compatible with a spreadsheet application such as Microsoft® Excel® Software.
      - *.csv: comma-separated value-file
3. For each file, select the data to be exported.
   a. Under the Properties & Data tab, check the properties and data to be exported.
   b. Under the Fold Change Results tab, check the properties and data to be exported.
4. Select/deselect **Open after exporting**, then select **Export**.

If **Open after exporting** has been selected, the software will open a *.txt file in a text editing program, and it will attempt to open the *.csv file in your default spreadsheet software.
Open and view the exported files

1. Right-click on the exported file, then select Open With > Microsoft Office Excel to view the contents as a spreadsheet.
2. Use the AutoFit function in Excel to make the data easier to view.
   a. Click in the upper-left corner of the spreadsheet to select all cells.
   b. Select Format > Column > AutoFit Selection.

Troubleshooting failed analysis

An error message will appear if the experiment files have not been set up properly. Below are some common set up errors that may occur. Refer also to “Analysis rules” on page 17.

- Make sure that the correct Reference Use is selected in the Edit Study Property screen:
  o Per Plate: Each assay must have a reference sample assigned in the same plate.
  o Per Study: Each assay must have a reference sample assigned, but it can be in a different plate within the same study.
- Each plate must have at least one NPC assigned per assay.
- Each sample-assay combination must be unique across the study. The same sample/assay combination cannot be assigned to multiple plates.
- Each assay-sample combination must be assigned a task, either "Unknown," "Reference," or "NPC."
- Every assay must have at least one unknown sample.
Appendix A. About TaqMan® Protein Assay data analysis

About TaqMan® Protein Assays

TaqMan® Protein Assay reagents enable detection and relative quantitation of protein targets in mammalian cell culture samples using an adapted form of PLA™, a proximity ligation assay technology, in combination with real-time PCR (Figure 1).

Figure 1. TaqMan® Protein Assays: proximity ligation with real-time PCR

Total protein cell lysates are prepared, and a dilution series of each lysate is incubated with paired assay probes. These probes consist of antibodies for the protein of interest conjugated to 5’ and 3’ oligonucleotides. The ends of the oligonucleotides are brought into proximity when the antibody components of the assay probe pair concurrently bind to two different epitopes on the target protein. A bridge structure can then form by hybridization of a third oligonucleotide to the assay probe oligonucleotide ends. This structure is captured through ligation, and the ligation product is then amplified and detected by TaqMan real-time PCR. For further information, see the TaqMan® Protein Expression Assays Chemistry Guide (PN 4405780).

How TaqMan® Protein Assay data analysis differs from traditional real-time PCR analysis

The Cₚ data from TaqMan Protein Assays reflect not only the real-time PCR but also the assay probe binding and ligation events. Therefore relative quantification of protein targets with TaqMan Protein Assays uses a different approach from relative quantification of mRNA or DNA targets via traditional real-time PCR. For a description of the fold change algorithm, see Appendix B.
Key features of TaqMan Protein Assay data analysis

- **C_T values are normalized to cell count or total protein concentration**, because no suitable endogenous controls are currently available.
- **C_T values from buffer-only control samples (no-protein control samples; NPC)** that are run on the same plate are **used to correct for background ligation** that occurs in the absence of protein. The C_T value corrected for background ligation is called ΔC_T.
- **A dilution series of each sample is used**, and the **linear range within this series is identified** and used to estimate the quantity of target protein in the sample relative to the quantity of this protein contained by a reference sample. For a drug or chemical treatment study, the reference sample could be untreated cells; for a time course study, time-zero cells.
- **The differences in the slope and intercept of the regression lines for the samples** are incorporated into the fold change algorithm, to account for the differences in assay efficiencies associated with the protein-antibody binding dynamics.
Appendix B. Fold change algorithm

The following provides information about how fold change values are estimated. The fold-change estimate is intended to estimate the amount of a target protein contained in a target cell type or cell state relative to that contained in a reference cell type or cell state. It is applicable to situations where the amount of target protein per cell cannot be determined but the exact number of cells processed by the assay can be determined.

Linear regression is applied to data from the linear regions of the dilution curves for the unknown and reference samples. These operations yield slope and intercepts for each of the samples, $A_{unk}$, $B_{unk}$, $A_{ref}$, and $B_{ref}$ respectively. The fold change estimate is given by the following equation:

$$F = b \left( \frac{B_{unk} - Q_T}{A_{unk}} \right) / \left( \frac{B_{ref} - Q_T}{A_{ref}} \right)$$

where $Q_T$ is the quantification threshold value that you have chosen and $b$ is the base of the logarithm used to transform the input quantity and, hence, the base of the logarithm used in the linear regression operation. The equation is based on an exponential model relating protein quantity to the production of ligation product coupled with the governing equation relating fluorescence and the quantity of ligation product fed into the PCR.

The validity of the fold-change estimate relies on the following key assumptions:

1) The average per-cell content of the target protein is a reasonable value to characterize the per-cell content over the sample of cells processed; i.e., the distribution of per-cell protein content is assumed monomodal over the population of cells analyzed.

2) The spontaneous formation of ligation product in the absence of the target protein is independent of the context of molecular structures associated with the protein in its natural state (e.g., cellular debris when examining target protein content in a cell of interest). In other words, it is assumed that the characteristics of the spontaneous formation of ligation product is completely captured by examining the case where no cells are input to the assay, the NPC as described for this product.

3) The value of $Q_T$ is appropriate for the unknown and reference samples.

4) In the case where references and/or unknown samples are spread across two or more plates, it is assumed that the NPC also accounts for the major factors underlying plate-to-plate variability.