ProteinAssist™ Software

Data analysis software for TaqMan® Protein Assays

Getting Started Guide

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About this guide

Purpose

This guide is intended to help you quickly learn to use ProteinAssist™ Software to analyze your TaqMan® Protein Assays data.

- You can use this guide as a reference.
- You can also follow the structured tutorial exercises using the example data files provided with the software. See "Tutorial overview" on page 17.

Prerequisites

This guide assumes that you have working knowledge of:

- Procedures for performing TaqMan Protein Assays.
- Microsoft® Windows® XP operating system.
- Instrument operation and system software for your Real-Time PCR System.

Install ProteinAssist™ Software

System requirements

Table 1 Hardware configuration and operating system requirements

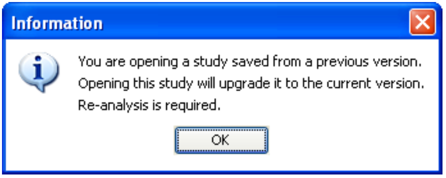
Minimum hardware configuration	Recommended hardware configuration
Intel CPU, 2.4 GHz	Intel CPU, 3.0 GHz
Available hard disk space: 1 GB	Available hard disk space: 20 GB
Memory: 1 GB	Memory: 2 GB
Screen resolution: 1024 × 768 pixels	Screen resolution: 1280 × 1024 pixels
Operating System: Windows® XP 32-bit (recommended Service Pack 3)	

Note: ProteinAssist™ Software has not been verified on system configurations other than the two listed in Table 1.

Install the software

1. Go to **www.appliedbiosystems.com/proteinassist**, and follow the prompts for downloading the ProteinAssist™ Software.
2. Navigate to the folder containing the downloaded installer, and double-click **setup.exe** to start the installation.
3. Follow the prompts to install the ProteinAssist Software in the desired location.

   Note: If you are re-installing or upgrading ProteinAssist™ Software, you must first uninstall the software (see page 8). The uninstall process protects existing data.
4. (Optional) If you are re-installing or upgrading ProteinAssist™ Software:
   - Install the software on the same drive on which the previous version was installed.
   - You will be prompted to restore any data from the previous version of the software. Click **Yes**.
   - The first time you open a study that was saved in a previous version of the software, you will be prompted to re-analyze the study. Click **OK**.



Uninstall ProteinAssist™ Software

Uninstall the software

1. Open **Windows Control Panel ▸ Add or Remove Programs**.
2. Select **ProteinAssist™** Software, click **Change/Remove**, and follow the prompts to uninstall the software.

   Note: The uninstall process protects existing data in the background.

(Optional) Save data before an uninstall

If desired, you can use the **Transfer out Study** tool to save your ProteinAssist Software studies outside of the application workspace before you uninstall the software (refer also to "Transfer out a study" on page 55):

1. In the Home window, select a study, and click **Transfer out Study**.
2. Navigate to the desired folder, enter a name for the study file (*.las), then click **Save**.
3. Follow "Transfer a study into ProteinAssist™ Software" on page 19 to restore the saved files, if necessary.

2 Set up ProteinAssist™ Software

Overview

After ProteinAssist™ Software is installed, set up the software:

- Familiarize yourself with how data is managed in the ProteinAssist™ Software (this page), and the software workflow (page 11)
- Set the default file save location (page 11) and adjust the default study settings, if necessary (page 12)
- Familiarize yourself with key toolbars and software conventions (page 14)

About ProteinAssist™ Software

ProteinAssist™ Software performs relative quantification calculations with your TaqMan® Protein Assay C_T 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. See Appendix A on page 61 for information about TaqMan Protein Assays, key differences in data analysis of TaqMan Protein Assays and traditional real-time PCR, and the algorithm used to calculate fold change (relative quantification).

About studies, experiment files, and samples

In the ProteinAssist Software, data is managed as a study.

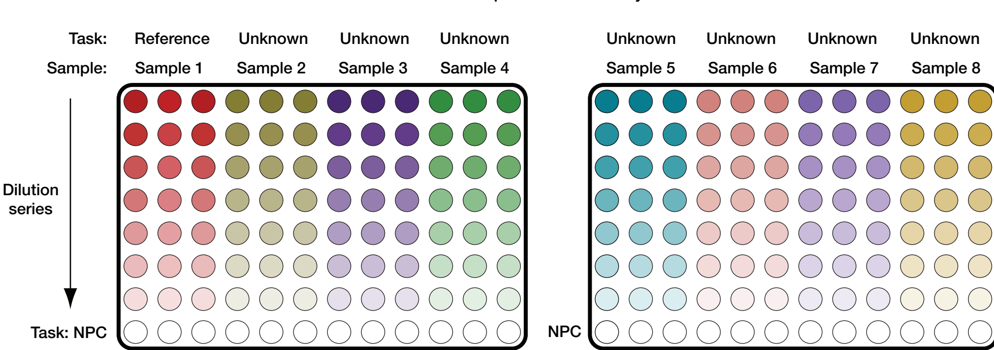
- A study is a collection of TaqMan Protein Assay experiment files.
- An experiment file contains TaqMan Protein Assay data from a single reaction plate.
  - The plate must be from one of the Applied Biosystems real-time PCR systems listed in Table 2 on page 10.
  - Each experiment file must have a unique file name.
- A sample includes all the wells in a dilution series of one lysate. For detailed information, refer to *TaqMan® **Protein Assays** Sample Prep and Assay Protocol* (PN 4449283).

A study allows you to analyze data from multiple reaction plates. Optionally, one reference sample can be used in analysis of all the experiment files for that TaqMan Protein Assay.

We recommend analysing studies with no more than two hundred 96- or 384-well plates.

Figure 1 shows an example TaqMan Protein Assay plate layout for the dilution series for 8 different lysates, with sample names and task assignments that could be assigned in the software. With the plate setups shown in Figure 1, the C_T data for each plate is imported into ProteinAssist Software as an experiment file. Sample 1 is the reference sample for samples 2 through 8. The data from both plates is analysed as a single study, and the Reference Use would be set to Per Study.

Figure 1 TaqMan® Protein Assays: samples and task assignments



Compatible real-time PCR systems

Table 2 Compatible real-time PCR systems and plate files

Real-time PCR system (Fast system recommended)	Plate file extension	System software
7500 Fast system	*.eds, *.txt	7500 Software v2.0.2, v2.0.3, and v2.0.4
	*.csv	SDS Software v1.4
7900HT/7900HT Fast system • Standard 96-Well Block Module • 384-Well Block Module • Fast 96-Well Block Module	*.txt	SDS Software v2.3 Patch A, B, C
StepOnePlus™ system	*.eds, *.txt	StepOne™ Software v2.1
ViiA™ 7	*.eds, *.txt	ViiA™ 7 Software v1.0 RUO only

ProteinAssist™ Software Workflow

Set up a study (Chapter 3)

Launch a study

Set required study properties: Study Name, Reference Use

Set up the experiment files within a study (Chapter 4)

Import TaqMan® Protein Assay plate files

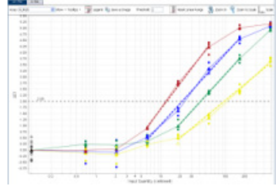
Assign required well properties: Sample, Assay, Task, Input Quantity

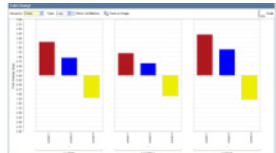
Analyze the study (Chapter 5)

Review data for each dilution series

Adjust analysis settings (optional) and omit outliers, if desired

View fold change results as a bar graph or heat map





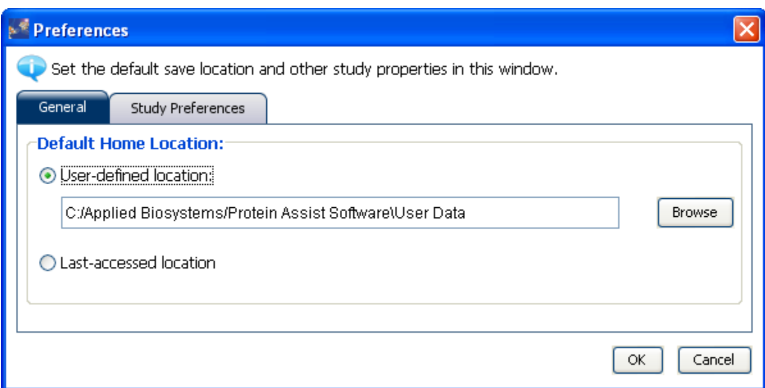
Save or export study data (Chapter 6)

Set default save location

To set the default location for saving exported data and study files:

1. In the Home window, select **Tools** ▸ **Preferences** ▸ **General** tab.

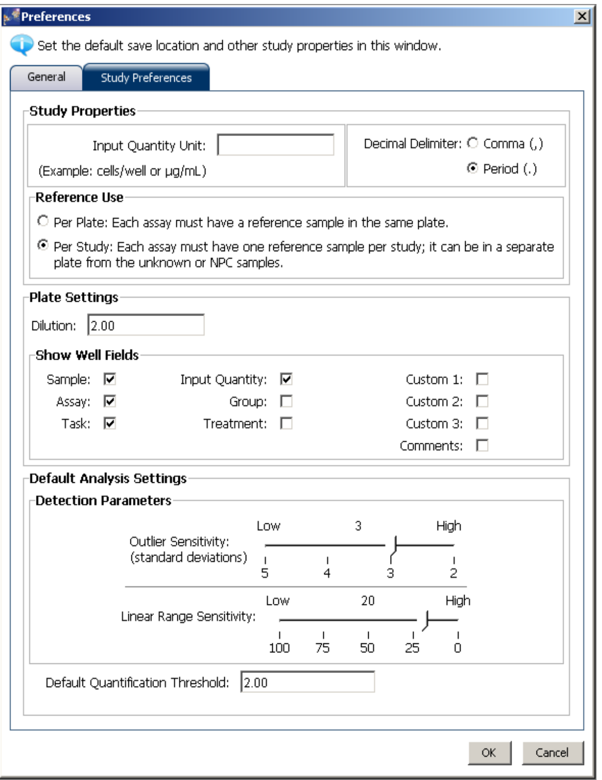
2. Select from the following options:
   - **User-defined location**: Click **Browse** to navigate to your selected folder.
   - **Last-accessed location**: The folder you last visited, in or out of the application.



(Optional) Adjust study preferences

Adjust study preferences to match study parameters you use most commonly.

1. In the Home window, select **Tools** ▸ **Preferences** ▸ **Study Preferences** tab.



2. Adjust the study preferences, if necessary, using the information in Table 3 on page 13.

Table 3 Study Preferences

Setting	Purpose	Action or comments
Input Quantity Unit (optional setting)	Sets the units to be displayed in the data plots and at other points in the software.	Enter the units of the input quantities for the samples. Default is blank. The units can be set for individual studies. See page 19.
Decimal Delimiter	Sets the format of numerical values in exported *.txt files.	Select period (.) or a comma (,) as the decimal delimiter. Default is period.
Reference Use	Sets how the reference sample is used for data analysis.	Choose a setting: • **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. Default is Per Study. The setting can be changed for individual studies.
Dilution	Sets the default dilution factor for the Dilution tool in the Plate Layout tab. (This tool allows you to easily assign input quantities to a dilution series by a click-and-drag function.)	Enter the dilution factor most commonly used in your TaqMan® Protein Assays. Default value is 2.0. The dilution factor can be adjusted for individual experiment files in the Plate Layout tab. See page 33.
Show Well Fields	Sets the well fields (properties) to be displayed in the Plate Layout screen and the Well Editor.	Check the desired well fields. Default setting is Sample, Assay, Task and Input Quantity, which are properties required for analysis. The setting can be adjusted for individual studies. See page 26.
Outlier Detection	Sets the sensitivity of the software for flagging outlier wells in the Plate Layout and the Well Table. The lower the sensitivity, the more an individual well must deviate from its replicate group's average C_T value to be named an outlier.	Set the standard deviations from the average of replicate C_T values. Default setting is 3. The setting can be adjusted for an individual study. See page 51. **Note:** Flagged outliers are included in data analysis unless manually excluded using the Omit tool. See page 51.

Setting	Purpose	Action or comments
Linear Range Detection	Determines the sensitivity of the linear range algorithm that 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 ΔC_T values of replicate groups must be colinear. The regression line of the data within the linear range of the dilution curve for each sample is automatically computed when you click **Analysis** in the Analysis toolbar. For more information, see "Linear range algorithm" on page 62.	Adjust the sensitivity of the algorithm with the slide tool: • **High sensitivity:** A replicate group is considered part of the linear range only if its average ΔC_T 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 ΔC_T value deviates significantly from the regression line of an initial set of two or three replicate groups chosen by the algorithm. Default setting is 20. The setting can be adjusted for an individual study. See page 49.
Quantification Threshold	The Quantification Threshold is a factor used in the fold change algorithm. See "Fold change algorithm" on page 62.	Default value is 2.0. See Appendix B on page 67 for information on adjusting the Quantification Threshold.

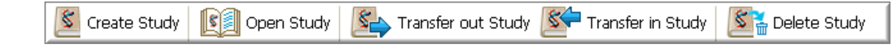
Key toolbars and software conventions

Home window toolbar

The Home window displays upon launching ProteinAssist Software. Use the home window tools to:

- Create new studies, open existing studies, or transfer a study file into the application workspace (page 19).
- Transfer studies out of the application workspace in a ProteinAssist Software-compatible format (page 55).
- Delete studies from the application workspace (page 56).

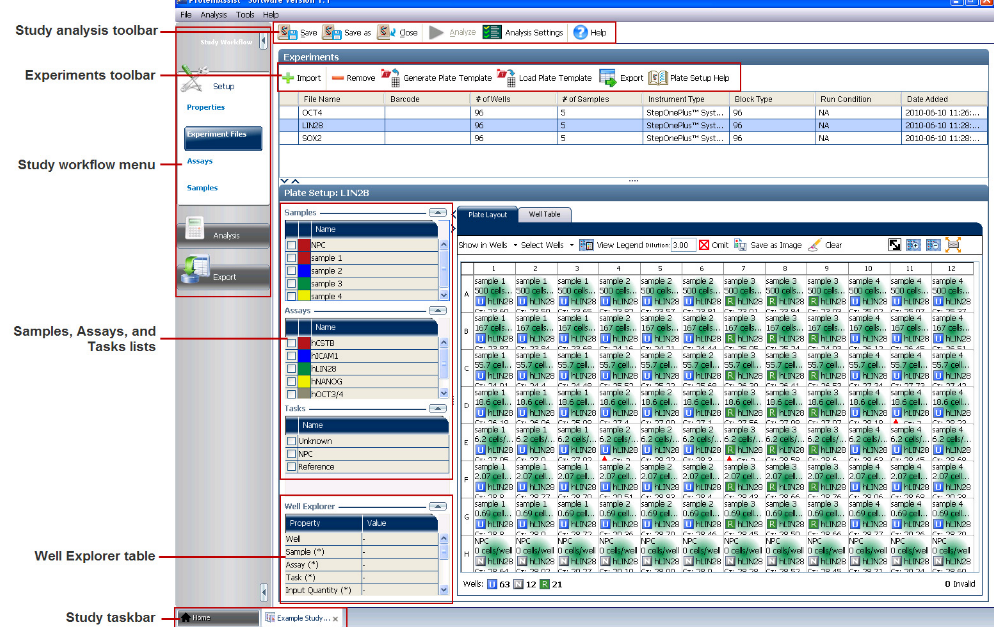
Figure 2 Home window toolbar



Study workflow menu

Use the study workflow menu to access the Setup, Analysis, and Export functions of the software for a given study (Figure 3 on page 15).

Figure 3 Key toolbars and menus



Study analysis toolbar

These tools manage data at the study level (Figure 3) . The same study analysis toolbar is accessible in all the Setup, Analysis, and Export screens. Use these tools throughout the software workflow to:

- Analyze, save, or close a study.
- Adjust the study analysis settings.
- Access this guide (click **Help**).

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

Experiments toolbar

These tools manage data at the experiment file level (Figure 3). Use the Experiments toolbar to:

- Import experiment files into a study.
- Remove experiment files from a study.
- Generate a plate template from an experiment file.
- Apply a previously generated plate template to an experiment file.
- Export the plate settings and data in a spreadsheet-compatible format.

For further information, refer to Chapter 4.

Study taskbar

Use the study taskbar at the bottom of the application window to quickly switch between open studies and the home window (Figure 3).

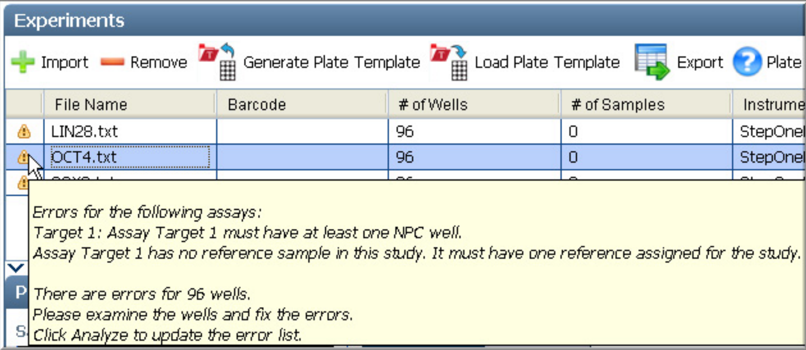
Samples, Assays, and Tasks lists, and Well Explorer table

These tools are located to the left of the Plate Layout tab in the Experiment Files window (Figure 3) . Use these tools to view and adjust well properties in an experiment file. For further information, refer to Chapter 4.

Field error icon

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

Figure 4 Field error icon tooltip display



Set up a study

Overview

To set up a study:

1. Launch a study one of the following ways (page 19):
   - Create a new study
   - Open an existing study
   - Transfer a ProteinAssist™ Software-compatible study file (*.las) into the application workspace
2. Set the study properties (page 19)
3. (Optional) Manage study assays (page 21)
4. (Optional) Manage study samples (page 22)

Tutorial overview

Several files with example data are included with ProteinAssist Software (Table 4 on page 18). You can use these files in structured tutorial exercises that are included at key places in this guide.

The example data represents what might be seen in a study to examine the effects of an inducer and an inhibitor of expression of the pluripotency markers OCT3/4, LIN28, and SOX2 in an experimental cell line.

Figure 5 on page 18 illustrates the layout for each reaction plate in the example study.

- Each plate has one TaqMan® Protein Assay: hLIN28, hOCT3/4, or hSOX2.
- Each plate has the following samples:
  – 1 reference sample for that assay (no treatment).
  – 2 samples that were treated with inducer.
  – 1 sample that was treated with inhibitor.
- The dilution factor for the samples depends on the assay:
  – LIN28: 3-fold
  – OCT4: 2-fold
  – SOX2: 3-fold

Figure 5 Example Study v2.0 plate layout

Task: Unknown Unknown Reference Unknown
Treatment: Inducer Inducer Control Inhibitor
Sample: Sample 1 Sample 2 Sample 3 Sample 4
Dilution series
Task: NPC

Table 4 lists the example data files that are installed by the software at C:\Applied Biosystems\Protein Assist Software\User Data\examples.

Table 4 Example data files provided with ProteinAssist™ Software

File	Description	Use
Example Study v2.0.las	A complete study file with data that is already set up for analysis in the software.	In the tutorial, the study file is transferred into the application workspace (see page 23). You can use this ready-made study to explore features of the software.
LIN28 v2.0.txt OCT4 v2.0.txt SOX2 v2.0.txt	Plate files that contain C_T data from the StepOnePlus™ instrument software.	In the tutorial, each plate file will be imported into ProteinAssist™ Software and set up as an experiment file that includes other well property assignments.
Template_LIN28 v2.0.lpt Template_OCT4 v2.0.lpt Template_SOX2 v2.0.lpt	Template files for the corresponding .txt files.	You can apply the template files to the corresponding .txt files in ProteinAssist™ Software to quickly generate experiment files that are ready for analysis (see page 39). *- or -* Follow the structured tutorial exercises to: • Set up the LIN28 experiment file with the LIN28 template file (page 39). • Set up the OCT4 (page 34) and SOX2 (page 40) experiment files using other ProteinAssist™ Software tools. The structured tutorial exercises create a study, Tutorial_Study, that is identical to Example Study v2.0 after the experiment files have been set up for analysis.

Launch a study

Launch a study in one of the following ways.

Create a study

In the Home window toolbar, select **Create Study**. The Setup ▸ Properties window automatically opens.

Open a study

In the Home window toolbar, highlight a study in the list, then click **Open Study**. The Setup ▸ Properties window automatically opens.

Transfer a study into ProteinAssist™ Software

ProteinAssist Software stores studies as internal files in the application workspace. These internal files are not accessible to users. The Transfer in/out Study tools allow study files to be shared outside of the application workspace (such as, by e-mail) in a ProteinAssist Software-compatible format. Study files are first transferred out of the application workspace (using the Transfer out Study tool) and saved as independent files with a .las file extension (see page 55).

To access a study file that was created in another ProteinAssist Software application:

1. In Home window toolbar, select **Transfer in Study**.
2. Navigate to the location of your saved study file (*.las).
3. Select the file, then click **Open** to transfer in the files.
4. Highlight the study in the studies list, then click **Open Study**. The Setup ▸ Properties window automatically opens.

Set study properties

The following steps, illustrated in Figure 6 on page 20, describe how to set the study properties for newly created studies.

1. In the Study Workflow menu, click **Setup ▸ Properties** to open the study properties window.
2. Enter the values in the property fields or select the appropriate option. These properties are required:
   - Study Name
   - Reference Use

   Table 5 on page 20 describes all the study properties available through the study properties window.
3. In the study analysis toolbar, select **Save**.

   The study is saved in the application workspace. It can be accessed through the Home window.

Figure 6 Study Properties window

Table 5 Study properties

Property or field	Entry
Study Name (required)	Enter a name for the study, maximum 100 characters. The study name is incorporated into the default name(s) for files created by the **Export** and **Transfer out Study** functions.
Input Quantity Unit (optional)	Enter the units describing the concentration of your samples. The input quantity units display in the data plots and at other points in the software.
Reference Use (required)	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.

Property or field	Entry
Custom Field Names (optional)	Enter custom field names that you may need to manage your studies, maximum 30 characters. **Note:** Custom field names cannot be empty. If you do not assign custom field names, leave the default values (Custom 1, etc.) in the fields. Example: Set a custom field name of "Time." When you set up the experiment files, enter the time associated with each sample in this custom field. **Note:** Custom field values are sorted as text. Enter time values in a format that can be sorted as text. For example, Day_01, Day_05, Day_10, etc.
Description (optional)	Enter a short description of the study, maximum 160 characters. The description displays in the study list in the Home window.
Comments (optional)	Enter comments, then select **Add**. The software records the comments and the date and 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).

(Optional) Manage assays

You can manage assays as described in this section, or when you set up the experiment files, described in Chapter 4.

In the Study Workflow menu, click **Assays** to open the Assays window, shown in Figure 7. The six assays commercially available from Applied Biosystems are preloaded for your convenience. Table 6 on page 22 describes the assay management features of the software.

Figure 7 Assays window

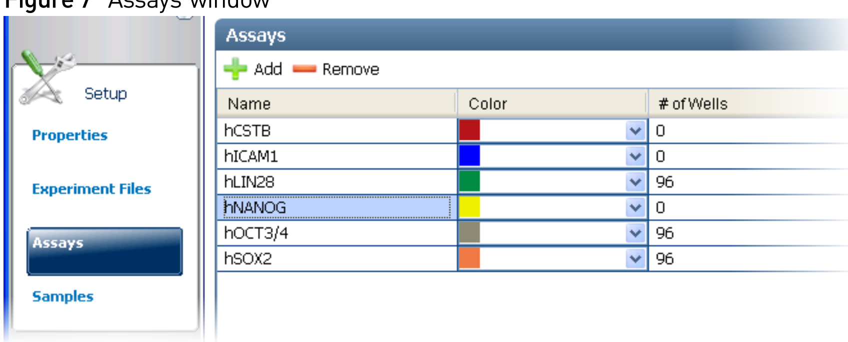


Table 6 Assays management

To...	Action
Add new assays	Click **Add** and type the new assay name.
Change the color of an assay (the assay color is displayed in the Plate Layout tab)	Select from the Color drop-down menu.
Remove assays that are not used in the study.	Select the assay (ctrl-click or shift-click to select multiple assays) and click **Remove**. Assays in use cannot be removed.

(Optional) Manage samples

You can manage samples as described in this section, or when you set up the experiment files, described in Chapter 4.

In the Study Workflow menu, click **Samples** to open the Samples window, shown in Figure 8. Table 7 describes the samples management features of the software.

Figure 8 Samples window

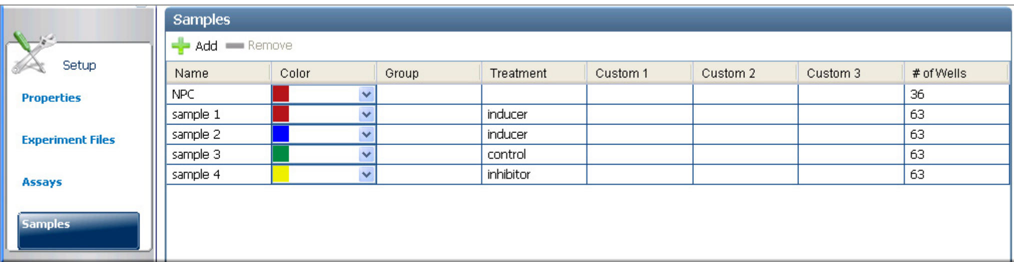


Table 7 Samples management

To...	Action
Add new samples.	Click **Add** and type the new sample name.
Change the color of a sample (displayed in the Plate Layout tab and in the Linear Range plots).	Select from the drop-down menu.
Edit Group, Treatment, or Custom properties for a sample.	Double-click in the field of interest for that sample and edit its value. **Note:** When the sample is assigned to wells in an experiment file, Group, Treatment, or Custom properties associated with that sample are automatically assigned to the wells.
Remove samples that are not used in the study.	Select the sample (ctrl-click or shift-click to select multiple samples) and click **Remove**. Samples in use cannot be removed.

Tutorial: transfer in Example Study

To transfer in Example Study v2.0.las, follow "Transfer a study into ProteinAssist™ Software" on page 19, navigating at step 2 to:

C:\Applied Biosystems\ProteinAssist Software\User Data\examples

- The example 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," because each plate has one TaqMan Protein Assay, and the reference sample for each assay is on the plate.

Tutorial: create Tutorial_Study and set its properties

1. In the Home window toolbar, select **Create Study**.
2. In the Properties window, enter the following tutorial study properties:
   - Study Name: **Tutorial_Study**
   - Input Quantity Unit: **cells/well**
   - Reference Use: **Per Plate**
3. In the study analysis toolbar, select **Save**.

After experiment files have been added to the study and saved, the study contents area in the study properties window displays the number of experiment files, assays, and samples in the study.

Set up experiment files

Overview

After you have launched a study, set up experiment files for analysis as follows.

1. Import TaqMan® Protein Assay experiment files from your real-time PCR instrument software (page 25).
2. Assign required and optional properties to the wells in each experiment file.
   - Required properties: sample, assay, input quantity, and task.
   - ProteinAssist™ Software provides a number of flexible tools for assigning well properties:
     - Well Editor (page 30)
     - Dilution tool (page 33)
     - Plate Templates (page 37)
     - Copy and Paste tools (page 37)
     - Samples, Assays, and Tasks lists (page 38)
     - Well Explorer tool (page 38)

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

In the tutorial exercises, the example experiment files provided with the software, LIN28, OCT4, and SOX2, are set up for analysis, each using a different set of ProteinAssist Software tools.

Import experiment files into a study

About importing experiment files

In ProteinAssist™ Software, an experiment file contains TaqMan Protein Assay data from a single reaction plate, and it is equivalent to a real-time PCR instrument plate file. This guide uses the term *experiment file*. ProteinAssist Software is compatible with real-time PCR instrument experiment files as described in Table 2 on page 10. For some real-time PCR instruments, the experiment file may include sample name, input quantity, or assay name, in addition to the C_T values.

- Only .eds, .txt or .csv files can be imported.
- Each experiment file must have a unique name.

IMPORTANT! Changing the content of .txt or .csv files before importing them into ProteinAssist Software may prevent importing the files, or it may result in incorrect analysis in ProteinAssist Software.

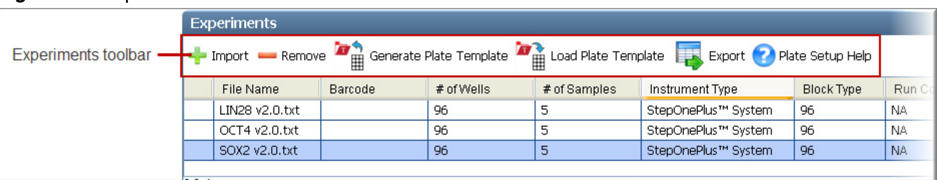
Import experiment files

Figure 9 illustrates the following procedure for importing experiment files.

1. Launch a study, then click **Setup** ▸ **Experiment Files** in the Study Workflow menu to open the Experiment Files window.
2. In the Experiments toolbar, click **Import** and navigate to the file(s) to be imported.
3. In the Import dialog window, select the appropriate file type from the drop-down menu, select the file name(s) and click **Import**.

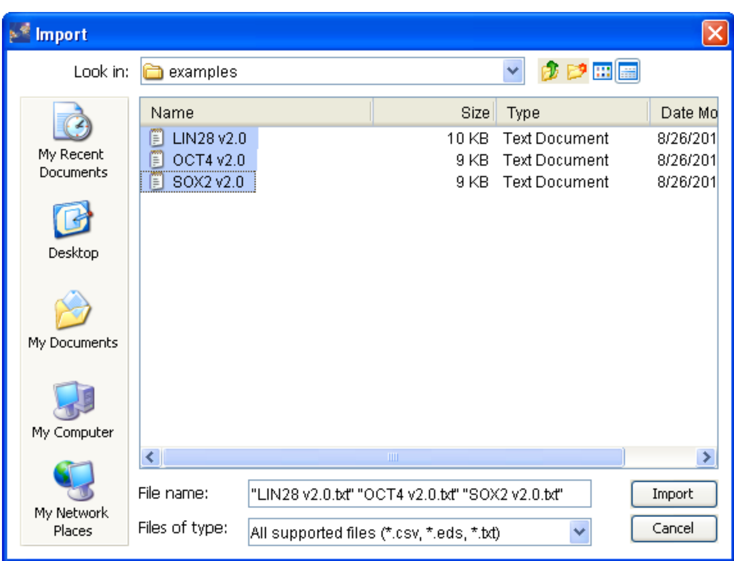
   Note: Ctrl- or shift-click to select and import multiple files.

Figure 9 Experiments Files window and toolbar



Tutorial: import the LIN28, OCT4, and SOX2 experiment files

1. In the Home window, open Tutorial_Study.
2. In the Study Workflow menu, click **Setup** ▸ **Experiment Files**.
3. In the Experiments toolbar (see Figure 9), click **Import** and navigate to C:\Applied Biosystems\Protein Assist Software\User Data\examples.
4. In the Import dialog window, ctrl-click to select **LIN28 v2.0.txt**, **OCT4 v2.0.txt**, and **SOX2 v2.0.txt**, then click **Import**.



About viewing experiment files and selecting wells

Experiment files can be viewed as a plate layout or as a table.

View data in the Plate Layout tab

Figure 10 on page 27 shows an example of a plate layout grid. Outlier wells are flagged with a red triangle; to exclude outliers from data analysis, see "Omit outlier wells" on page 51. Table 8 on page 27 describes the tools available in the Plate Layout toolbar for changing the display and data in the plate layout view.

Figure 10 Plate Layout view

Table 8 Plate Layout toolbar

Tool	Description
Show in Wells	• Select data to show or hide in the wells. • Differentiate samples or assays by color, or choose to have no color-coded display in the wells.
Select Wells	Select wells by well property: assay, sample, task, group, treatment, or custom property.
View Legend	Displays the well legend in the plate layout grid.
Dilution	Automatically creates a dilution series from an initial input quantity. See page 33.
Omit or include wells from data analysis	For selected wells, click to omit from *or* include in data analysis. **Note:** Omitted wells are designated with a cross in the plate layout grid and a * in the well table. See also page 51.
Save as Image	Saves the plate layout grid as an image file. See Table 7 on page 22.
Clear	Clears the properties of the selected well, excluding C_T value.
Full Screen	Expands the plate layout grid to the screen size. Click the icon again to reset.
Zoom in or out	Step-zooms in or out of the plate layout grid view.
Fit plate	Fits the plate layout grid in the current window.

About selecting wells in the Plate Layout tab

In the Plate Layout tab, select wells in one of the following ways. See also Figure 10 on page 27.

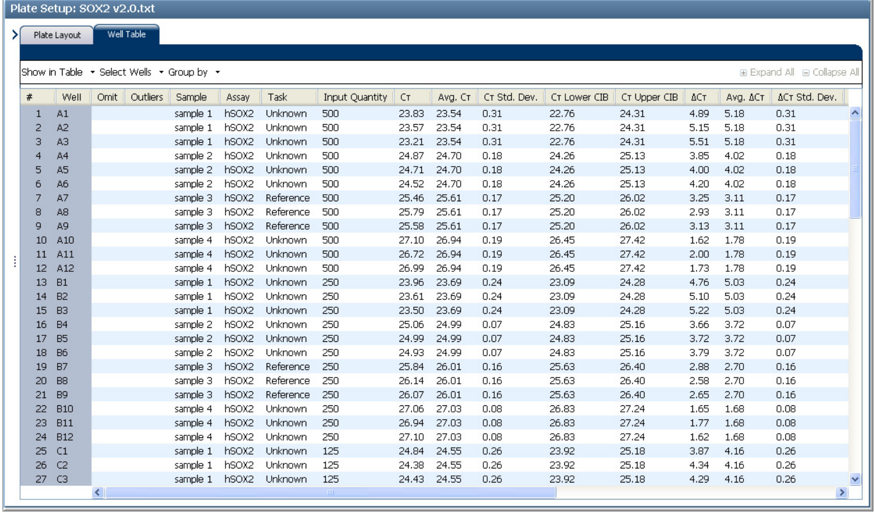
Table 9 Selecting wells in the Plate Layout tab

To select...	Action
One or more wells	Click-drag through the desired wells. **Note:** When you click-drag to select multiple wells, ensure that the selection arrow not the cross-hair icon is displayed. (The cross-hair icon indicates you are using the Dilution tool, not selecting wells.)
An entire row	Click **A, B, C,....H**
An entire column	Click **1, 2, 3,.....12**
The entire plate	Select the top left outside corner of the plate grid.

View data in the Well Table tab

Click the Well Table tab to view the data and properties associated with the experiment file in a flexible table format, as shown in Figure 11. Outlier wells are flagged with a *. Table 10 on page 29 describes useful features of the well table and tasks that can be performed in the Well Table tab, including manual omission of outlier wells.

Figure 11 Well Table view



#	Well	Omit	Outliers	Sample	Assay	Task	Input Quantity	CT	Avg. CT	CT Std. Dev.	CT Lower CIB	CT Upper CIB	ΔCT	Avg. ΔCT	ΔCT Std. Dev.
1	A1			sample 1	hSOX2	Unknown	500	23.83	23.54	0.31	22.76	24.31	4.89	5.18	0.31
2	A2			sample 1	hSOX2	Unknown	500	23.57	23.54	0.31	22.76	24.31	5.15	5.18	0.31
3	A3			sample 1	hSOX2	Unknown	500	23.21	23.54	0.31	22.76	24.31	5.51	5.18	0.31
4	A4			sample 2	hSOX2	Unknown	500	24.87	24.70	0.18	24.26	25.13	3.85	4.02	0.18
5	A5			sample 2	hSOX2	Unknown	500	24.71	24.70	0.18	24.26	25.13	4.00	4.02	0.18
6	A6			sample 2	hSOX2	Unknown	500	24.52	24.70	0.18	24.26	25.13	4.20	4.02	0.18
7	A7			sample 3	hSOX2	Reference	500	25.46	25.61	0.17	25.20	26.02	3.25	3.11	0.17
8	A8			sample 3	hSOX2	Reference	500	25.79	25.61	0.17	25.20	26.02	2.93	3.11	0.17
9	A9			sample 3	hSOX2	Reference	500	25.58	25.61	0.17	25.20	26.02	3.13	3.11	0.17
10	A10			sample 4	hSOX2	Unknown	500	27.10	26.94	0.19	26.45	27.42	1.62	1.78	0.19
11	A11			sample 4	hSOX2	Unknown	500	26.72	26.94	0.19	26.45	27.42	2.00	1.78	0.19
12	A12			sample 4	hSOX2	Unknown	500	26.99	26.94	0.19	26.45	27.42	1.73	1.78	0.19
13	B1			sample 1	hSOX2	Unknown	250	23.96	23.69	0.24	23.09	24.28	4.76	5.03	0.24
14	B2			sample 1	hSOX2	Unknown	250	23.61	23.69	0.24	23.09	24.28	5.10	5.03	0.24
15	B3			sample 1	hSOX2	Unknown	250	23.50	23.69	0.24	23.09	24.28	5.22	5.03	0.24
16	B4			sample 2	hSOX2	Unknown	250	25.06	24.99	0.07	24.83	25.16	3.66	3.72	0.07
17	B5			sample 2	hSOX2	Unknown	250	24.99	24.99	0.07	24.83	25.16	3.72	3.72	0.07
18	B6			sample 2	hSOX2	Unknown	250	24.93	24.99	0.07	24.83	25.16	3.79	3.72	0.07
19	B7			sample 3	hSOX2	Reference	250	25.84	26.01	0.16	25.63	26.40	2.88	2.70	0.16
20	B8			sample 3	hSOX2	Reference	250	26.14	26.01	0.16	25.63	26.40	2.58	2.70	0.16
21	B9			sample 3	hSOX2	Reference	250	26.07	26.01	0.16	25.63	26.40	2.65	2.70	0.16
22	B10			sample 4	hSOX2	Unknown	250	27.06	27.03	0.08	26.83	27.24	1.65	1.68	0.08
23	B11			sample 4	hSOX2	Unknown	250	26.94	27.03	0.08	26.83	27.24	1.77	1.68	0.08
24	B12			sample 4	hSOX2	Unknown	250	27.10	27.03	0.08	26.83	27.24	1.62	1.68	0.08
25	C1			sample 1	hSOX2	Unknown	125	24.84	24.55	0.26	23.92	25.18	3.87	4.16	0.26
26	C2			sample 1	hSOX2	Unknown	125	24.38	24.55	0.26	23.92	25.18	4.34	4.16	0.26
27	C3			sample 1	hSOX2	Unknown	125	24.43	24.55	0.26	23.92	25.18	4.29	4.16	0.26

Table 10 Well Table tools

To...	Action
Hide or reveal columns	Click the **Show in Table** drop-down menu in the Well Table tab toolbar and select or deselect the desired fields. Show in Table
Select all rows with the same well property	Click the **Select Wells** drop-down menu in the Well Table tab toolbar and select a property. Select Wells
Group rows by a well property	1. Click the **Group by** drop-down menu in the Well Table tab toolbar and select a well property. Group by 2. Select **Expand All** or **Collapse All** to expand or collapse the groups. Expand All Collapse All
Sort the data	Click the column header of interest. With each click, the sort toggles through ascending (shown), descending, and none. Sample ▲1
Perform multiple sorts	Click the first column header of interest, then ctrl-click the next column header of interest. Continue the sort by ctrl-clicking each column header of interest. In the example below, the table was sorted in this order: Sample (descending), Assay, Input Quantity (descending). Sample ▼1 Assay ▲2 Task Input Quantity ▼3
Re-order the columns	Click a column header and drag it to a new location in the table.
Omit or include a well from data analysis	Right-click the well, then select **Omit** or **Include**. Click **Analyze** in the study analysis toolbar to re-analyze the study. 47 D11 Copy Paste Omit Include To omit multiple outlier wells, click the **Outliers** column heading to sort the wells by outlier status, ctrl-click or shift-click to select all the outliers (flagged with a *), right-click and select **Omit**. Click **Analyze** as above.

Tutorial: Further exploration of data views

Open Example Study v2.0, and use the expand/collapse and resize tools (Figure 12 on page 30) to change the display of the Experiments list and Plate Setup window in the Experiment Files window.

Figure 12 Experiment Files window: expand/collapse and resize tools

Assign well properties

About assigning well properties

Experiment files from the instrument software are incomplete for analysis. All experiment files within a study must be assigned with required well properties, and they must follow the analysis rules in order for analysis to proceed.

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

Analysis rules

These analysis rules can also be accessed by clicking **Plate Setup Help** in the Experiments toolbar. Plate Setup Help

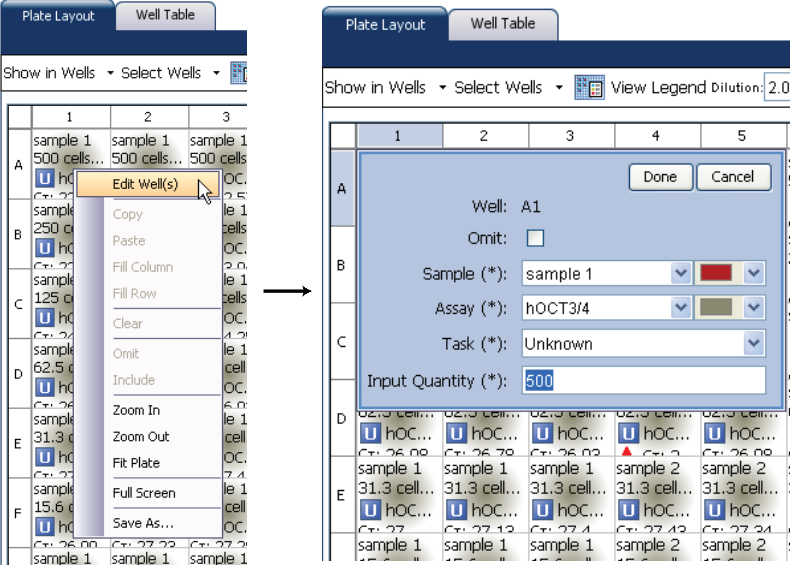
- The study must have wells to analyze. It does not support cases where all wells are omitted.
- All included wells must have Sample, Assay, Task and Input Quantity specified.
- Each assay-sample combination must be assigned with only one task, either "Unknown," "Reference," or "NPC."
- Each assay-sample combination with a task assignment of “Unknown” must be unique across the study.
- Every assay-sample combination must have at least two input quantities.
- Every experiment file must have at least one NPC well for each assay assigned in the experiment.
- Every assay must have at least one unknown sample.
- If Reference Use is Per Study: Every assay must have one reference sample, but it can be in a different experiment file within the same study.
- If Reference Use is Per Plate: Every assay must have one reference sample in the same experiment file.

Assign well properties using the Well Editor

1. In the Study Workflow menu, click **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-drag to select multiple wells.
3. Right-click to access the well menu (Figure 13, left) and select **Edit Well(s)** display the Well Editor (Figure 13, right).

   (You can also access the Well Editor for a single well by selecting the well and either double-clicking the mouse or pressing the **Enter** key.)

Figure 13 Well menu and Well Editor tool



4. Enter values into the required fields: **Sample**, **Input Quantity**, **Task**, and **Assay**. See Table 11.
5. (Optional) Assign other properties to samples for sorting and display purposes (Table 11).
   a. Click **Show in Wells** in the Plate Layout toolbar, and select one or more of the following optional fields: **Group**, **Treatment**, **Custom 1**, **Custom 2**, **Custom 3**. The selected field(s) appear in each well and in the edit well window.
   b. Select wells and enter the field values as described in steps 2 through 4.
6. Select **Done** to exit the Well Editor and save the well assignments.

Table 11 Entering well property values using the Well Editor tool

Well Property	To Assign a Value...	Notes
Sample (required)	Enter a new sample name. *-or-* Select a sample name from the drop-down menu.	Sample names are imported from the instrument experiment files if sample names were assigned within the instrument software. Sample names automatically appear in the Samples list to the left of the Plate Layout tab after you enter a new sample name or import an instrument experiment file with pre-assigned sample names. **Note:** A sample includes all the TaqMan Protein Assay wells in the dilution series for one cell lysate. If you assigned multiple sample names in the instrument software (for instance, one sample name per well), re-assign a single sample name to the appropriate wells in the ProteinAssist™ Software, and delete the unused sample names. See Troubleshooting starting on page 59 for further information.
Input Quantity (required)	Enter a numeric input quantity for the sample.	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.
Task (required)	Select **Reference**, **Unknown**, or **NPC**.	Reference: A reference sample is required for each assay, and it is assigned per plate or per study (see Table 5 on page 20). Unknown: Test sample. NPC: No Protein Control. There must be at least one NPC per assay per plate. **Note:** When you assign NPC, the well is automatically assigned a sample name of NPC and an input quantity of 0. Other well properties are cleared.
Assay (required)	Select an assay from the drop down menu. *-or-* Enter a new assay name.	Target names are imported as Assays from instrument experiment files if target names were assigned within the instrument software. Assay names automatically appear in the Assays list to the left of the Plate Layout tab after you enter a new assay name or import an experiment file with pre-assigned target names. ProteinAssist™ Software has 6 assays pre-loaded for your convenience: hCSTB, hICAM1, hLIN28, hNANOG, hOCT3/4, and hSOX2
Group (optional)	Enter the value.	This property is useful for describing variables such as sample handling or source.
Treatment (optional)	Enter the value.	Examples: inducer, inhibitor, none.

Well Property	To Assign a Value...	Notes
Custom 1, 2, 3 (optional)	Enter the value.	To rename Custom 1, Custom 2 and Custom 3, go to **Setup ▸ Properties** and enter a new name. **Note:** Custom field names cannot be empty. If you do not assign custom field names, leave the default values (Custom 1, etc.) in the fields. **Note:** If you are using a custom field for time, enter time values in a format that can be sorted as text. For example, Day_01, Day_05, Day_10, etc.

Set up a dilution series with the Dilution tool

You can use the Dilution tool to set up a dilution series after the highest Input Quantity (and optionally other well properties) has been assigned to the well(s), as described below and in Figure 14. This tool is similar to a click-and-drag autofill function in spreadsheet software.

1. Enter the desired dilution factor in the **Dilution** field of the Plate Layout toolbar.
2. Select one or more wells.
3. Hover the pointer over the lower right corner of the selected well(s) until the dilution tool 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 is reduced by the dilution factor in each succeeding well, and other well properties, excluding C_T value, from the first well, row, or column are copied into the selected wells.

IMPORTANT! The Dilution tool copies other well properties (except C_T value) from the first well, row, or column into the selected wells, overwriting previous well property assignments.

In the example shown in Figure 14, row A was selected, then the Dilution tool was used to create the dilution series for all wells through row G.

Figure 14 Create a dilution series with the Dilution tool

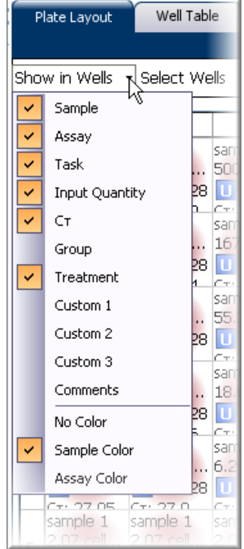
Tutorial: set up the OCT4 experiment file using the Well Editor and Dilution tool

This tutorial exercise demonstrates two ways to use the Well Editor and Dilution tool to set up an experiment file.

- Use the Well Editor to assign all well properties for samples 1 and 2.
- Use the Well Editor to assign the well properties to the first well in the dilution series for samples 3 and 4, then use the Dilution tool to assign the well properties to the rest of the wells in each dilution series.

1. Assign the assay.
    a. Open Tutorial_Study, click **Setup** ▸ **Experiment Files**, then select **OCT4 v2.0** from the list of imported files.
    The Plate Layout tab for the OCT4 experiment file displays. This experiment file initially has only the C_T value assigned for each well.
    b. Assign the hOCT3/4 assay to all the wells: select all the wells in the plate layout, right-click and select **Edit Well(s)** to access the Well Editor, and select **hOCT3/4** in the Assay drop-down menu. (Do not assign other well properties.)
2. Adjust the plate layout view. Click **Show in Wells** in the Plate Layout toolbar (Figure 15 on page 34), and:
    - Select **Treatment** so that it appears in the Well Editor window.
    - Select **Sample Color** to display sample assignments by color.

Figure 15 Show in Wells tool



3. Use the Well Editor to assign *all* the well properties for samples 1 and 2 as described in the following table: select the indicated wells, right-click and select **Edit Well(s)**, then enter the values as described in the following table. See Figure 13 on page 31.

Well Property	Value
Sample	• Columns 1–3, rows A–G: **Sample 1** • Columns 4–6, rows A–G: **Sample 2**
Input Quantity	• Columns 1–6, *row A only*: **500** • Columns 1–6, *row B only*: **167** • Columns 1–6, *row C only*: **55.7** • Columns 1–6, *row D only*: **18.6** • Columns 1–6, *row E only*: **6.2** • Columns 1–6, *row F only*: **2.07** • Columns 1–6, *row G only*: **0.69**
Task	Columns 1–6, rows A–G (samples 1 and 2): **Unknown**
Treatment	Columns 1–6, rows A–G (samples 1 and 2): **inducer**

4. Use the Well Editor to assign all the well properties for samples 3 and 4, including input quantity, to the *first* wells in the dilution series, as described below. See Figure 13 on page 31.

Well Property	Value
Sample	• Columns 7–9, *row A only*: **Sample 3** • Columns 10–12, *row A only*: **Sample 4**
Input Quantity	Columns 7–12, *row A only*: **500**
Task	• Sample 3 (columns 7–9), *row A only*: **Reference** • Sample 4 (columns 10–12), *row A only*: **Unknown**
Treatment	• Sample 3 (columns 7–9), *row A only*: **control** • Sample 4 (columns 10–12), *row A only*: **inhibitor**

5. Use the Dilution tool to simultaneously assign the input quantities and copy the other well properties to the rest of the wells for samples 3 and 4. See Figure 14 on page 33.
   a. In the Dilution field of the Plate Layout toolbar, enter: **3**.
   b. Select row A for samples 3 and 4 only (columns 7–12; Input Quantity = 500).
   c. Hover the pointer over the lower right corner of the selected wells until the Dilution tool crosshair appears. Drag the crosshair down to include rows A through G.

   The Input Quantity is reduced by the dilution factor, and other properties (excluding C_T value) are copied, when you use the Dilution tool. In this way, the well properties for samples 3 and 4 are assigned to rows B through G.

6. Set up the NPC samples: select row H (all columns), right-click to access the Well Editor, and select **NPC** from the Task drop-down menu.

   When a well is assigned the task NPC in the Well Editor, it is automatically assigned a sample name of NPC and an input quantity of 0.

Result

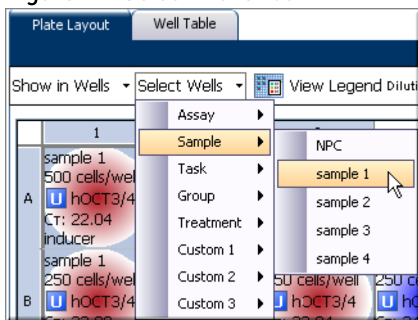
After you have completed assigning the well properties to the OCT4 experiment file, the plate layout grid should resemble Figure 16:

Figure 16 Tutorial_Study: OCT4 plate setup

Tutorial: Further exploration of the Plate Layout tab

Use the Select Wells tool to select wells by well property (Figure 17).

Figure 17 Select Wells tool



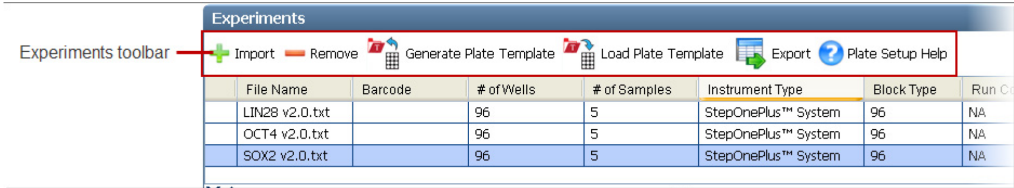
(Optional) Alternate methods for assigning well properties

Use a plate template

Use a previously generated plate template to assign well properties for the entire plate. This feature is useful when your studies use the same plate layout from experiment to experiment. Refer to page 42 for information about generating plate templates.

1. Select **Setup ▸ Experiment Files**, select an experiment file from the list, and click **Load Plate Template** in the Experiments toolbar.

   You can select multiple files with ctrl-click or shift-click.



2. In the dialog window, select a plate template (*.lpt) and click **Load**.

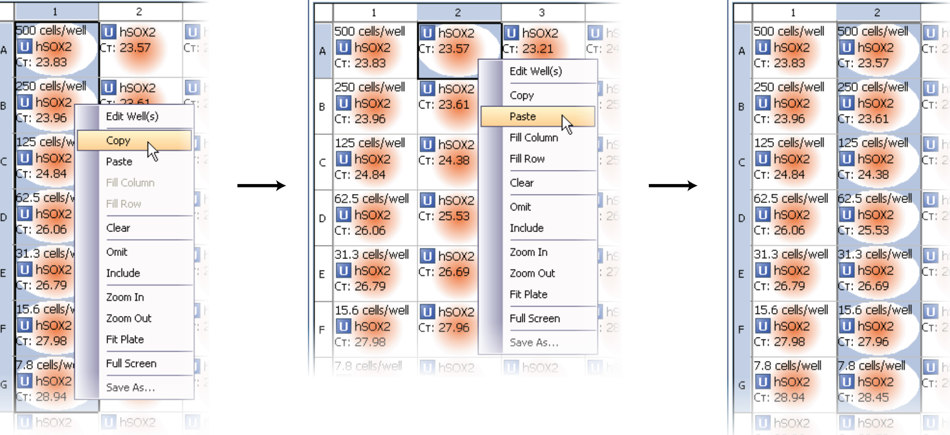
Use the Copy and Paste tools

Use the Copy and Paste tools in the well menu to copy all well properties, excluding C_T values, from one or more wells into other wells. You can also use these tools to copy properties from some or all of the wells in one plate to a different (or new) plate. This feature is similar to copy and paste functions in spreadsheet software.

1. In the Plate Layout tab, select the wells to be copied.
2. Right-click to access the well menu and select **Copy**.
3. Select the *top-left well* of the desired wells to copy to, right-click, and select **Paste**.

Figure 18 shows the properties from wells A-1 through G-1 being copied to wells A-2 through G-2.

Figure 18 Copy from multiple wells to multiple wells

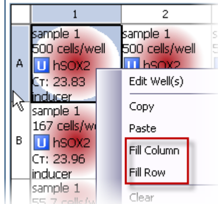


Use the Fill Column and Fill Row tools

Use the Fill Column and Fill Row tools to copy the properties of the selected well, excluding C_T value, and paste them into the remaining wells in the column or row.

In the Plate Layout tab, right-click the selected well, then select **Fill Column** or **Fill Row** from the drop-down well menu.

Figure 19 Fill Column and Fill Row tools



Use the Samples, Assays, or Tasks lists

Use the Samples, Assays, or Tasks lists, located to the left of the Plate Layout tab, to assign these corresponding well properties to selected wells. Use the up/down arrows to expand/collapse the lists, and the icon to move the list displays up/down (Figure 20).

- The Samples and Assays lists are populated when sample or assay assignments are made, using any tool, to any of the experiment files in a study.
- Group, treatment, or custom properties that have been assigned to a sample are automatically assigned to wells when that sample is assigned.

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

In the example shown in Figure 20, wells A-1 through G-3 have been selected and assigned “sample 1” from the Samples list.

Figure 20 Assign well properties using the Samples, Assays, or Tasks lists

Use the Well Explorer tool

For an *individual* well, all well properties except well location, task, and C_T value can be changed using the Well Explorer tool, located to the left of the Plate Layout tab.

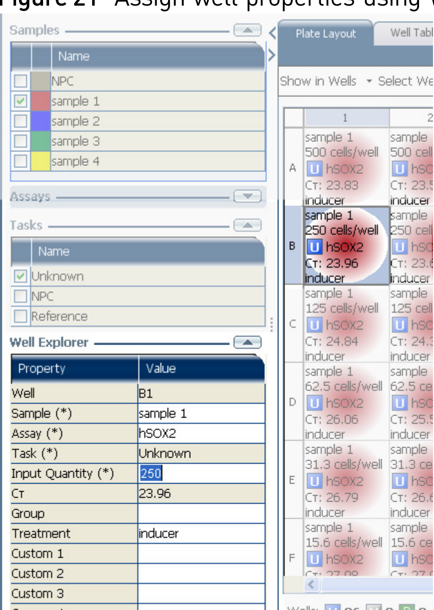
1. Select a single well. The assigned properties are displayed in the Well Explorer. Use the up/down arrow to expand/collapse the Well Explorer, and the icon to move the Well Explorer up/down.

2. Click the value for the property to be changed. If a property is editable, the value is highlighted.

3. Enter the value in the field. If you enter new values for sample and assay names, the Samples and Assays lists are updated.

In the example shown in Figure 21, all the well properties for well B-1 are editable except the well location, task, and C_T value.

Figure 21 Assign well properties using Well Explorer



Tutorial: Set up the LIN28 experiment file with a plate template

1. Open Tutorial_Study, click **Setup** ▸ **Experiment Files**, and select **LIN28 v2.0** from the list of imported files.

   The Plate Layout tab for the LIN28 experiment file displays. This experiment file initially has only the C_T value assigned for each well.

2. Click **Load Plate Template** in the Experiments toolbar.

3. In the Load window, navigate to C:\Applied Biosystems\Protein Assist Software\User Data\examples, select **Template_LIN28 v2.0.lpt**, and click **Load**.

4. Click **Show in Wells** in the Plate Layout toolbar (Figure 15 on page 34), and:

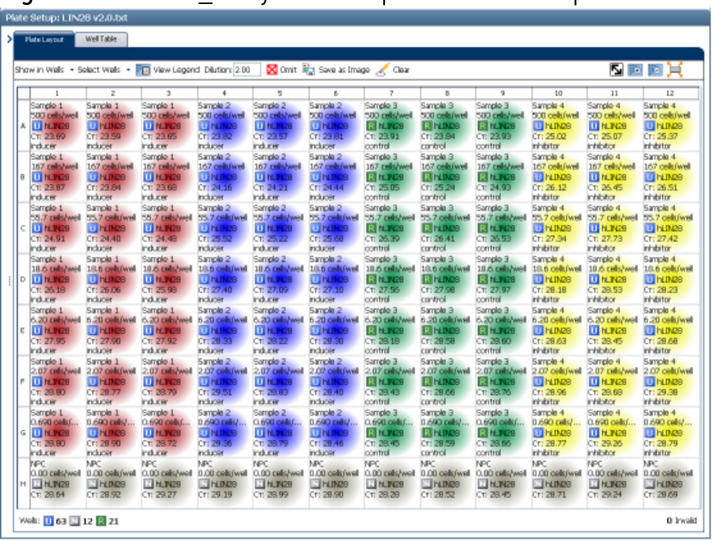
   a. Select **Treatment** so that it appears in the Well Editor window.

   b. Select **Sample Color** to display sample assignments by color.

5. Click **Save** in the Study Analysis toolbar.

The Plate Layout tab should look like Figure 22 on page 40.

Figure 22 Tutorial_Study: LIN28 experiment file setup



Tutorial: Set up the SOX2 experiment file with other tools

In this exercise, you use the Well Explorer, Copy and Paste tools, Dilution tool, and Samples, Assays, and Tasks lists to set up the SOX2 experiment file.

Note: Before proceeding with this tutorial, complete the tutorial exercise for OCT4 on page 34 or LIN28 on page 39, to populate the Samples, Assays, and Tasks lists.

1. Open Tutorial_Study, click **Setup ▸ Experiment Files**, and select **SOX2 v2.0** from the list of imported files.

   The Plate Layout tab for the SOX2 experiment file displays. This experiment file initially has only the C_T value assigned for each well, and a default task assignment "Unknown."

2. Click **Show in Wells** in the Plate Layout toolbar (Figure 15 on page 34), and:

   a. Select **Treatment** so that it appears in the Well Editor window.

   b. Select **Sample Color** to display sample assignments by color.

3. Assign the assay to all wells: click the top left corner of the plate layout grid to select all the wells, and check **hSOX2** in the Assays list.

4. Assign the initial input quantities in each dilution series using Well Explorer and copy/paste:

   a. In the plate layout grid, select well A-1.

   b. In the Well Explorer tool, enter **500** in the Input Quantity field.

   c. Right-click well A-1 and select **Copy** in the drop-down menu.

   d. Select wells 2–12 in row A, right-click and select **Paste**. The input quantity is copied to the rest of the row.

5. Assign input quantities to the rest of the wells using the Dilution tool. (Do this before assigning other well properties.)

   a. In the Dilution field, enter **3**.

b. Select row A (click **A**), hover the pointer in the bottom-right corner of the selected wells to activate the dilution cross-hair, and drag the cross-hair down through row G. See Figure 14 on page 33.

The input quantities are reduced by the dilution factor in series and assigned to the rest of the wells. Refer to “Set up a dilution series with the Dilution tool” on page 33).

6. Using the Samples list, assign samples to wells as shown in the following table. The Treatment field is automatically populated with the sample assignment.

Wells	Sample	Treatment
Columns 1–3, rows A–G	Sample 1	inducer
Columns 4–6, rows A–G	Sample 2	inducer
Columns 7–9, rows A–G	Sample 3	control
Columns 10–12, rows A–G	Sample 4	inhibitor

7. Assign the task using the Tasks list.

   a. Select all Sample 3 wells (columns **7–9**, rows A–G), and select **Reference** from the Tasks list.

   b. Select row H (all columns), and select **NPC** from the Tasks list.

8. Click **Save** in the Study Analysis toolbar.

When you are done, the plate layout grid should look like Figure 23:

Figure 23 Tutorial_Study: SOX2 experiment file setup

(Optional) Generate plate template

After the well properties 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 properties (such as Sample).

1. In the Experiments toolbar, click **Generate Plate Template**.
2. Enter a file name, navigate to the desired save location, then click **Generate** to save the new template.

Analyze a study

Overview

After the mandatory well properties have been assigned (sample name, assay name, task, and input quantity) to each experiment file in the study, following the analysis rules, the study is ready for analysis. Refer also to "Analysis rules" on page 30 for guidelines for assigning well properties.

1. Click **Analysis** in the Study Workflow menu.

   The software automatically calculates the fold change of the test samples with respect to the reference sample(s). The fold change algorithm corrects for background ligation in the absence of protein (ΔC_T) and factors in the linear range of each sample dilution series. For further information, see Appendix A on page 61.

2. Review the data for each dilution series in the Linear Range tool (starting on page 44).
   - Review the ΔC_T plot for all the samples
   - (Optional) Change the data and tooltips displayed in the ΔC_T plot
   - View the analysis results in table format
   - View the C_T data for troubleshooting purposes

3. Adjust the analysis settings (optional), omit outliers, if desired, and re-analyze the study (starting on page 48).
   - Review and adjust the Linear Range
   - Change the settings for outlier detection
   - Omit outliers, if desired

4. Review the fold change results (starting on page 52).
   - View the fold change results as a bar graph
   - View the fold change results as a heat map

Note: If the experiment files have not been set up correctly, an error message appears when you click **Analysis**. See "Troubleshooting" on page 59. Field error icons are cleared only after a study is successfully re-analyzed after you have corrected setup errors.

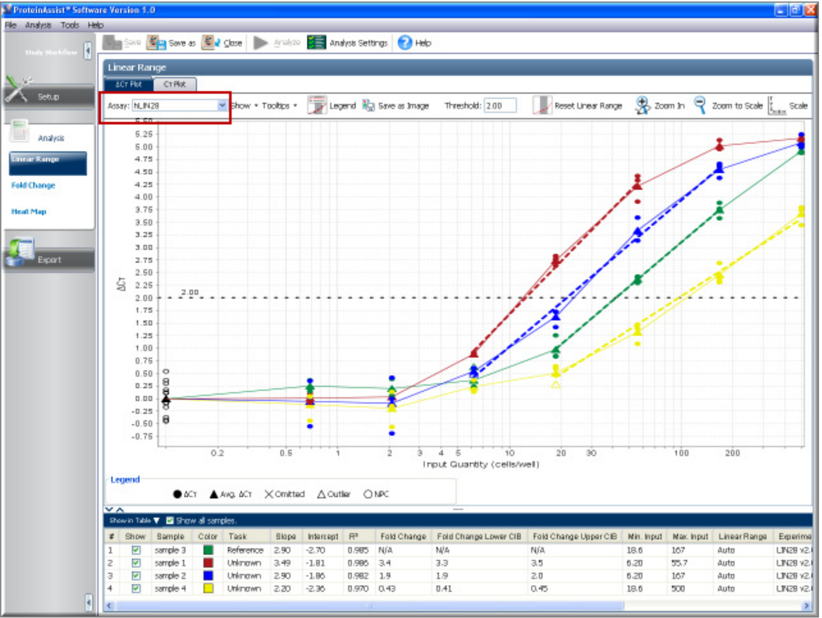
Note for tutorial: After you complete the tutorial exercises in Chapters 3 and 4, Tutorial_Study should be set up for data analysis. Alternatively, you can simply use Example Study v2.0, which is already set up for analysis, to help you explore the software analysis features (see "Tutorial: transfer in Example Study" on page 23).

Review the dilution series data in the Linear Range tool

1. Select **Analysis** ▸ **Linear Range** ▸ **ΔC_T Plot** tab.
2. In the ΔC_T Plot toolbar, select an assay from the drop-down Assay menu to view the ΔC_T results for that assay.

Figure 24 shows the ΔC_T plot and sample table for the hLIN28 assay in Example Study v2.0.

Figure 24 Linear Range tool: ΔC_T plot tab and sample table

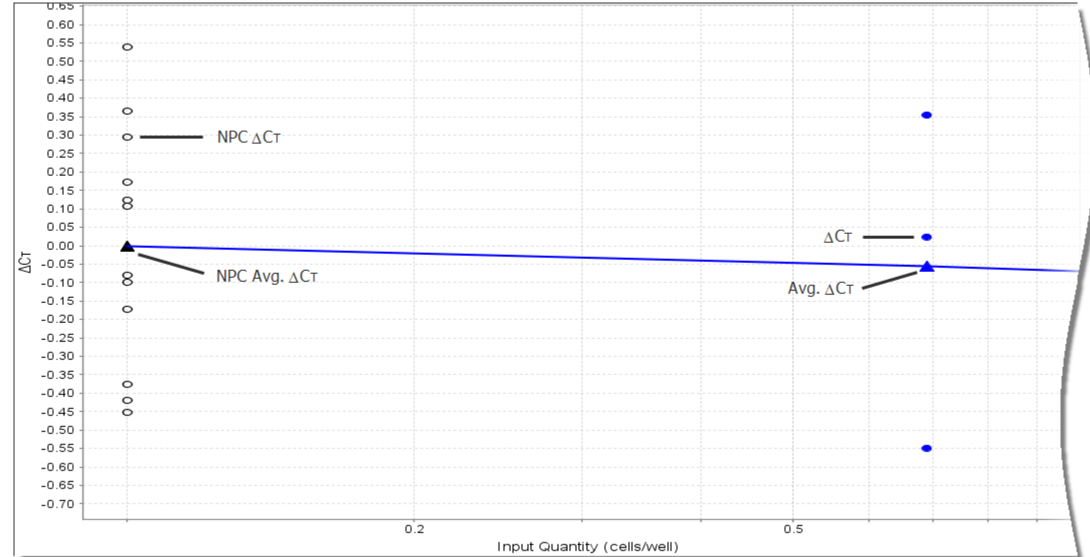


Review the ΔC_T plot

In the ΔC_T plot, the ΔC_T and average ΔC_T values for each sample-assay combination are plotted against the input quantities. In Figure 25, a portion of the ΔC_T plot has been enlarged to show an example of each of the data point styles.

- ΔC_T = average NPC C_T – sample C_T (solid colored circles)
- Average ΔC_T = average NPC C_T – average sample C_T (solid triangles)
- NPC ΔC_T = average NPC C_T – NPC C_T (open black circles)
- Average NPC ΔC_T = average NPC C_T – average NPC C_T (= 0; solid colored triangles)

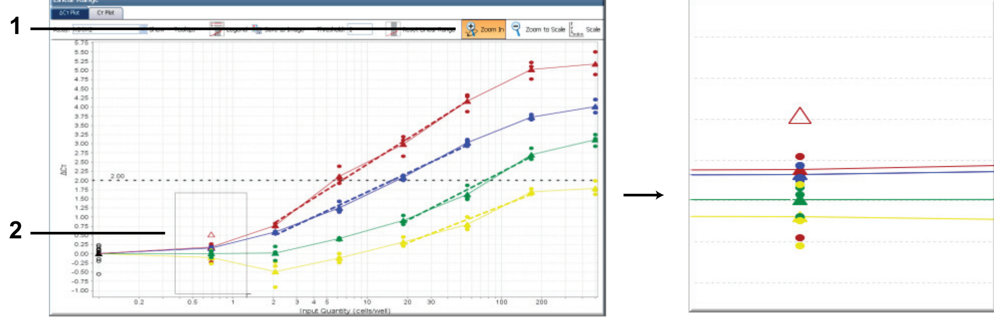
Figure 25 ΔC_T plot: data point styles



Zoom in to the ΔC_T plot

1. In the ΔC_T Plot toolbar, click **Zoom In**.
2. Click-drag around a region to enlarge.

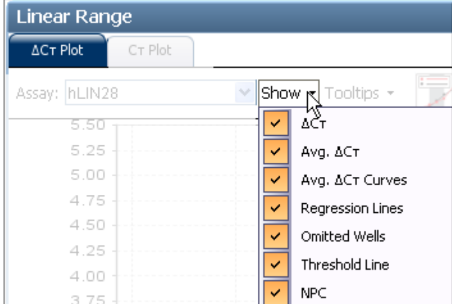
Figure 26 Zoom In tool



Change the data displayed in the ΔC_T plot

Click **Show** in the ΔC_T plot toolbar and select the desired items from the drop-down menu to show in the ΔC_T plot:

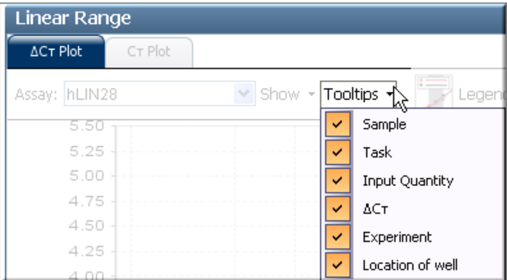
Figure 27 Show data in ΔC_T plot



Change the tooltips displayed in the ΔC_T plot

Click **Tooltips** in the ΔC_T Plot toolbar and select the desired well properties from the drop-down menu to show as tooltips in the ΔC_T plot:

Figure 28 Show tooltips in the ΔC_T plot



Review the sample table

The sample table, located below the ΔC_T plot (see Figure 24 on page 44), lists all the data and properties associated with each sample, as described in Table 12. Table 13 on page 47 describes useful tools for viewing data in the Sample Table tab.

Table 12 Key sample table columns

Column heading(s)	Description
Slope, Intercept, R^2	Values for the slope, intercept and R^2 of the regression line for each sample.
Fold Change	Fold change value for each sample-assay combination. • 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. • In some cases the fold change is reported as "Undetermined." See "Software rules for the fold change algorithm" on page 64.
Fold Change Lower/ Upper CIB	Lower and upper 95 % confidence interval boundaries, of the fold change value.
Min. and Max. Input	The minimum and maximum input quantity values of the linear range.
Linear Range	Linear range for this sample-assay combination was determined by: • Automatic setting • Manual setting • Unknown: the linear range algorithm failed to find a linear range. See "Troubleshooting" on page 59.
Task, Group, Treatment, Custom	Properties assigned to the sample-assay combination.

Table 13 Linear Range: tools for viewing data in the sample table

To...	Action
Sort the data in each column	Click on the column header.
Resize the columns	Hover the pointer between two column headings until the resize arrow ↔ appears, then drag the arrow.
Hide or show columns	Click **Show in Table** in the sample table toolbar, and select or deselect the fields in the drop-down menu.
Change the order of columns	Click-drag the column header.
Display or remove the sample ΔC_T curve from the ΔC_T plot	Check or uncheck the box in the **Show** column.

(Optional) Review the C_T plot

The C_T plot is useful for viewing the plotted NPC and uncorrected C_T values, for troubleshooting purposes. The C_T and average C_T values for each sample/assay combination are plotted against the input quantities.

- C_T values: solid circles
- Average C_T values: triangles
- NPC C_T: open black circles
- Average NPC C_T values: solid colored triangles

Note: You can change the data and tooltips displayed in the C_T plot in the same way as for the ΔC_T plot (see page 45).

Figure 29 C_T Plot tab

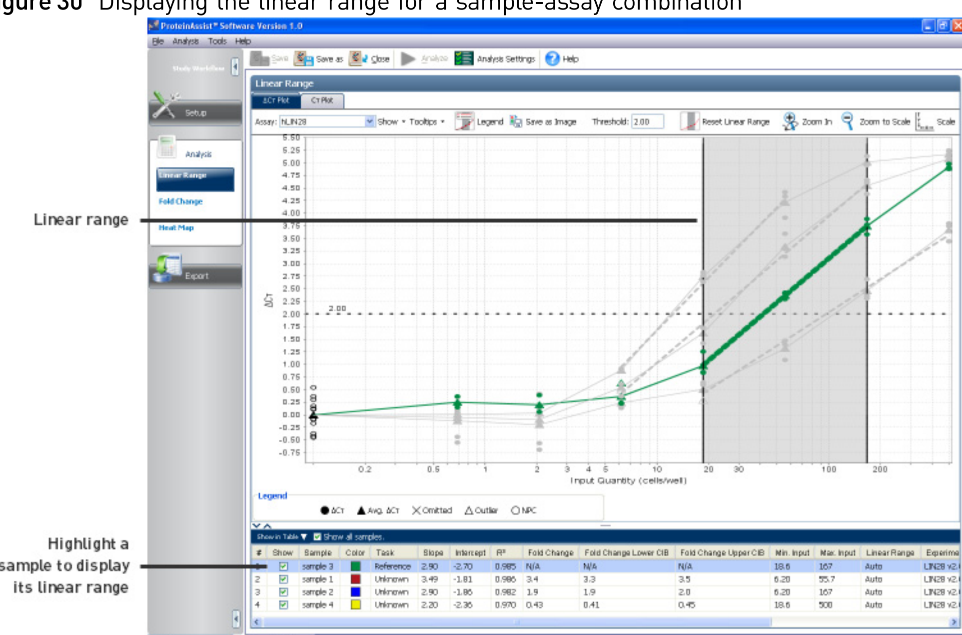
(Optional) Adjust the analysis settings and reanalyze the data

Review the linear range

When you click **Analysis** in the Analysis toolbar, ProteinAssist Software automatically determines a set of data for each sample that follows a linear relationship between log(input quantity) and ΔC_T. This set of data is used in the relative quantification (fold change) calculation. The zone encompassing the upper and lower boundaries of the data set is called the linear range.

- The linear range is displayed on the ΔC_T plot as a gray zone (see Figure 30).
- For each sample, a dotted line shows the regression line for that set of data.
- When you open the ΔC_T plot the first time for each study, the regression line and linear range for the reference sample are displayed.
- To display the linear range for a sample-assay combination, click the sample-assay combination in the sample table below the plot, or click the plot of the sample-assay combination.

Figure 30 Displaying the linear range for a sample-assay combination

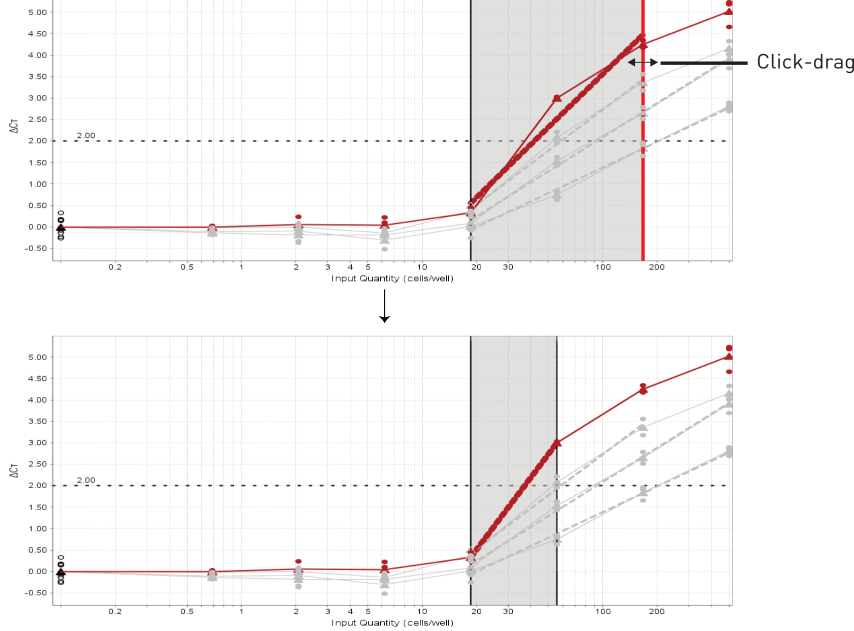


(Optional) Manually set the linear range of one or more samples

the following procedure describes how to manually adjust the data set used the calculate the regression line for a given assay-sample combination. Figure 31 on page 49 illustrates an example of an adjustment to the linear range so that it better reflects the transition region of the sigmoidal dilution curve. See "Linear range algorithm" on page 62 for further information.

1. Click the sample curve of interest in the ΔC_T plot or the sample row(s) in the table below the ΔC_T plot. The linear range for that sample displays as a gray zone.
2. Click-drag the left or right bar to adjust the upper and lower boundaries of the linear range.
3. Click **Analyze** in the toolbar for reanalysis.

Figure 31 Linear Range: viewing and adjusting the linear range

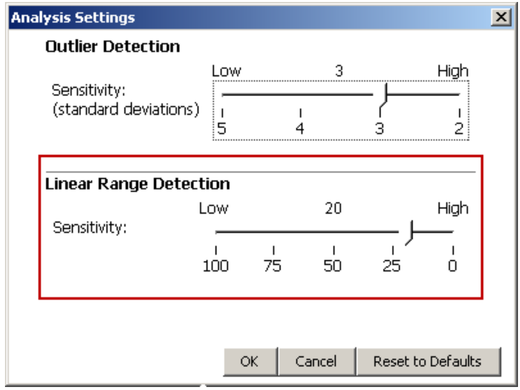


(Optional) Adjust the Linear Range Detection setting

The sensitivity of the linear range algorithm is the degree to which the average ΔC_T values of replicate groups must be colinear. The following procedure describes how to adjust the sensitivity for all samples in a given study while viewing the ΔC_T plot. Adjusting the sensitivity within a study overrides the default set for the software under **Tools** ▸ **Preferences** (Table 3 on page 13).

1. Click **Analysis Settings** in the Study Analysis toolbar.
2. Adjust the sensitivity of the algorithm with the slide tool (Figure 32 on page 50; default setting is 20):
   - **High sensitivity:** A replicate group is considered part of the linear range only if its average ΔC_T 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 ΔC_T value deviates significantly from the regression line of an initial set of two or three replicate groups chosen by the algorithm.
3. Click **OK**. The software automatically performs a re-analysis with the new setting(s).

Figure 32 Adjusting the Linear Range Detection setting



Tutorial: explore adjustments to linear range

1. Create a copy of a study, to explore adjustments to the data analysis settings: open Tutorial_Study or Example Study v2.0, click **Save as** in the study analysis toolbar, and enter a new file name.
2. Click **Analysis** ▸ **Linear Range** and select an assay of interest for display in the ΔC_T plot tab.
3. Select the Reference sample plot (highlight the Reference sample in the sample table), drag the lower boundary of the linear range to include one or more additional data points, and observe the new regression line in the ΔC_T plot.
4. Click **Analyze** in the Study Analysis toolbar, and observe the change in Fold Change values for the other samples in the study in the sample table.

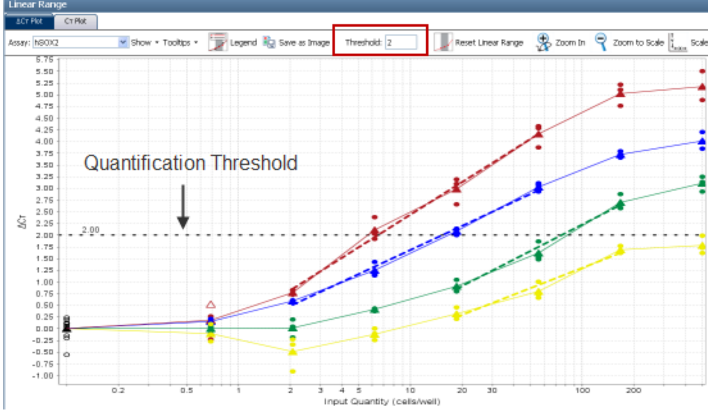
(Optional) Review the Quantification Threshold

The Quantification Threshold is shown on the ΔC_T plot as a horizontal dashed line (Figure 33). The Quantification Threshold:

- Is a factor used in the calculation for the fold change between the Unknown and Reference samples. See Appendix A on page 61.
- Has a default value of 2.0.

Improved accuracy of fold change values can be achieved by calibrating the Quantification Threshold as described in Appendix B on page 67.

Figure 33 ΔC_T Plot: Quantification Threshold



(Optional) Adjust Outlier Detection setting

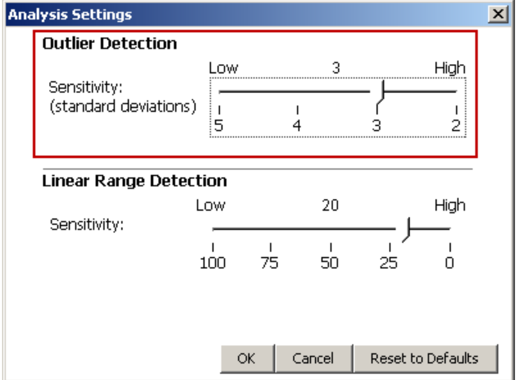
Outliers are automatically detected by ProteinAssist Software, and the individual wells are flagged on the ΔC_T plot as open triangles.

Note: Flagged outliers are included in data analysis unless they are manually omitted, as described in "Omit outlier wells", below.

It can be useful to adjust the Outlier Detection setting for the study while viewing the ΔC_T plot:

1. Click **Analysis Settings** in the Study Analysis toolbar.
2. Adjust the settings:
   - The lower the sensitivity, the more an individual well must deviate from its replicate group's average C_T value to be named an outlier.
   - Set the standard deviations from the average of replicate C_T values; default is 3 standard deviations.
3. Click **OK**. The software automatically performs a re-analysis with the new setting(s).

Figure 34 Adjusting the Outlier Detection setting



Omit outlier wells

Flagged outlier wells are included in data analysis unless they are manually excluded using the **Omit** tool. You can omit outlier wells in the following ways:

In the ΔC_T plot (illustrated in Figure 35 on page 52):

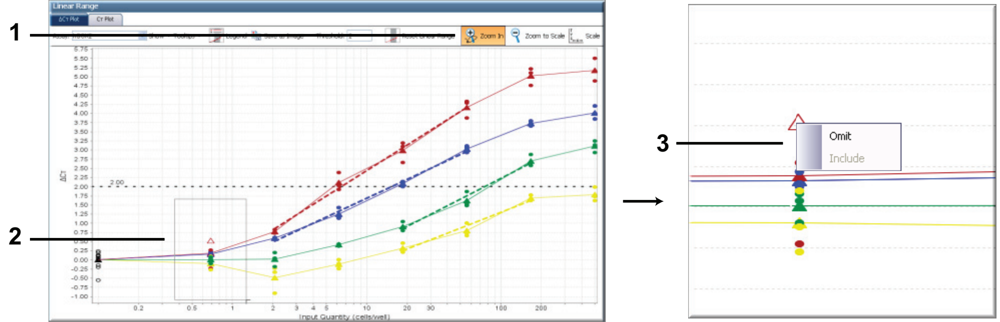
1. In the ΔC_T Plot toolbar, click **Zoom In**.
2. Click-drag around a region to enlarge (facilitates selecting outlier data points).
3. Select the outlier data point (open triangle) and:
   - **Omit the data point**: right-click on the outlier and select **Omit**.
   - **Include an omitted data point**: right-click on the data point and select **Include**.
4. Click **Analyze** in the study analysis toolbar to re-analyze the study.

In the C_T plot: follow the analogous procedure described above.

In the Setup ▸ Experiment Files ▸ Well Table tab (to omit all outliers in one batch): as described in Table 10 on page 29.

Note: A well with no C_T, $C_T \geq 40$, or $C_T \leq 0$ is automatically omitted by the software and it cannot be included.

Figure 35 Omit/Include outlier wells



View the fold change results as a bar graph

1. In the Study Workflow menu, click **Analysis ▸ Fold Change**.
   The fold change values for each sample-assay combination are displayed as a bar graph and as a table (Figure 36).
2. (Optional) Adjust the display of the bar graph as described in Table 14.

Figure 36 Fold change results: bar graph

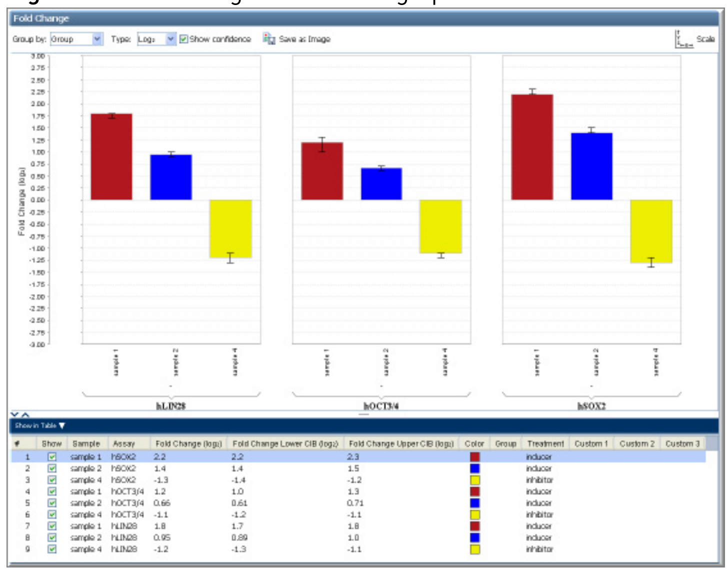


Table 14 Adjusting the fold change bar graph or sample table views

To...	Action
Change the fold change scale (Y-axis)	In the Fold Change toolbar: select **Log$_2$** (default), **Log$_{10}$**, or **Linear** from the **Type** drop-down menu. Type: Log2
Show or hide the 95% confidence intervals of the fold change results in the bar graph	In the Fold Change toolbar: check or uncheck **Show confidence**. Show confidence

To...	Action
Group the data in the bar graph by an additional well property (the software automatically groups the results by assay)	In the Fold Change toolbar: select a well property from the **Group by** drop-down menu. The property displays below the graph.
Show or hide a sample-assay combination in the bar graph	In the sample table: check or uncheck the box in the **Show** column.
Show or hide a well property or analysis result in the sample table	In the sample table: click **Show in Table** and check or uncheck the property or result in the drop-down menu.

Note: The fold change algorithm reports results in the range 10^{-6} to 10^{6}. For fold change values outside this range, the bar displays a break, and the fold change values are reported in the sample table as follows:

Fold change scale	Fold change <10^{-6}	Fold change >10^{6}
Log_2	<−20	>20
Log_{10}	<−6	>6
Linear	0.0	>1,000,000

View the fold change results as a heat map

The heat map display is useful when there are many assays and samples.

1. In the Study Workflow menu, select **Analysis ▸ Heat Map** (Figure 37 on page 54).
2. (Optional) Adjust the display of the heat map as described in Table 15 on page 54.

Figure 37 Fold change results: heat map view

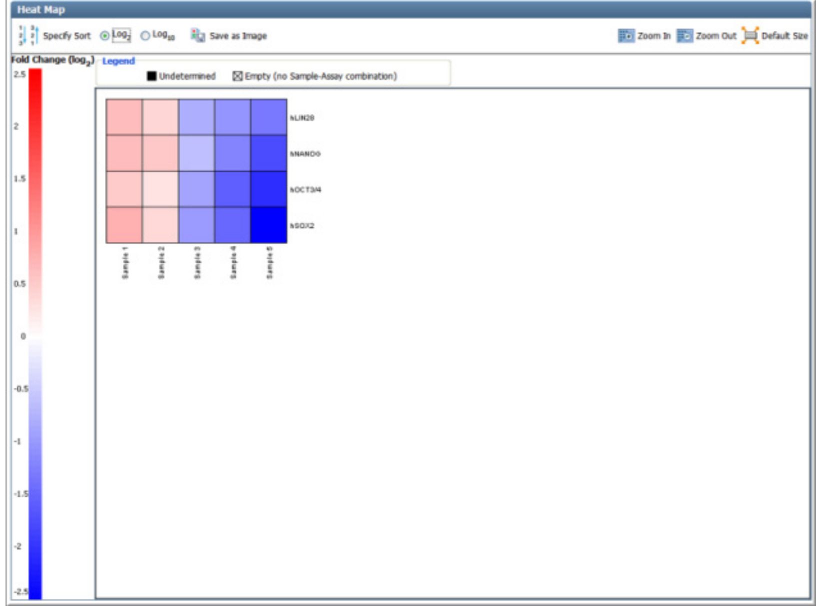


Table 15 Adjusting the fold change heat map view

To...	Action
Sort the data by one or more properties, in ascending order	Click **Specify Sort**, then select one or more sample properties in the Specify Sort Order window.
Change the fold change scale	Select **Log$_2$** (default) or **Log$_{10}$**.
Step zoom in/out	Click **Zoom In** or **Zoom Out**.
Restore the view to the default size	Click **Default Size**.

Note: The fold change algorithm reports results in the range 10^{-6} to 10^{6}. For fold change values outside this range, the sample color displays at the maximum value, reported on the heat map scale as follows:

Fold change scale	Fold change <10^{-6}	Fold change >10^{6}
Log$_2$	<–20	>20
Log$_{10}$	<–6	>6

Save or export study data

Overview

Study data can be exported or saved in a variety of formats, as described in Table 16.

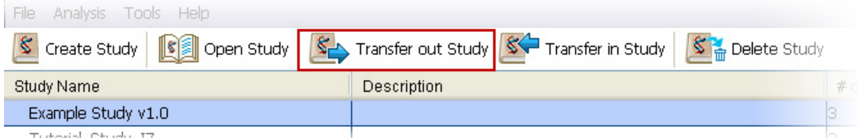
Table 16 Save and export options for study data

To...	File type	Menu commands
Save the plate layout grid as an image file	*.pdf *.png *.jpg	Setup ▶ Experiment Files ▶ Plate Layout tab ▶ Save as Image
Save the ΔC_T plot as an image file	*.pdf *.png *.jpg	Analysis ▶ Linear Range ▶ ΔC_T Plot tab ▶ Save as Image
Save the C_T plot as an image file	*.pdf *.png *.jpg	Analysis ▶ Linear Range ▶ C_T Plot tab ▶ Save as Image
Save the Fold Change bar graph as an image file	*.pdf *.png *.jpg	Analysis ▶ Fold Change ▶ Save as Image
Save the Fold Change heat map as an image file	*.pdf *.png *.jpg	Analysis ▶ Heat Map ▶ Save as Image
Save the study in the application workspace	Accessible only through the application	Study analysis toolbar ▶ Save or Save as
Save a study to a location outside the application workspace, in ProteinAssist™ Software-compatible format	*.las	See "Transfer out a study" below.
Export the selected plate setup (includes well properties and C_T data)	*.txt *.csv	Setup ▶ Experiment Files ▶ Export
Export the study data for analysis outside the ProteinAssist Software	*.txt *.csv	See "Export study data" on page 56.

Transfer out a study

The software stores studies as internal files in the application workspace. These internal files are not accessible to users. The **Transfer out Study** function allows you to move a study out of the ProteinAssist Software workspace, in a ProteinAssist Software-compatible format, to enable sharing with another user or for backup.

1. In the Home window, select the study of interest, then click **Transfer out Study**.



2. Browse to a save location, enter a name for the study file (*.las), then click **Save**.

   If an existing study file 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.

   ---

3. (Optional) After you have transferred out a study, you can share the study file with another user (for example, by e-mail). That user must then transfer in the study file to his or her application workspace. This process is described in "Transfer a study into ProteinAssist™ Software" on page 19.

(Optional) Delete a study

You can delete a study from the application workspace, without deleting the corresponding study file that has been transferred outside the application workspace as a *.las file.

In the Home window toolbar, select the study to be deleted, then click **Delete Study**.

Export study data

About exporting study data

The **Export** tool transfers 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 ΔC_T values, corresponding average values, standard deviation, and 95% confidence intervals for replicates.
- **Fold change results**: linear range and fold change data for each sample, as well as relevant sample properties.

Export a study

Figure 38 on page 57 illustrates the following procedure for exporting a study.

1. In the Study Workflow menu, click **Export** ▸ **Export**.
2. Choose an export option.
   - **Single File**: the well properties and data and the fold change results are exported to a single file.
   - **Separate Files**: the well properties and data are exported to one file, and the fold change results are exported to a separate file. For *each* export file, follow the rest of this procedure.
3. Set the location of the exported file: accept the default location, or select **Browse** to navigate to the desired save location.
4. Set the name and format of the exported file:

a. (Optional) Modify the default file name, if desired. 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.

- *.txt: tab-delimited text file
- *.csv: comma-separated value file

5. Select the data to be exported.

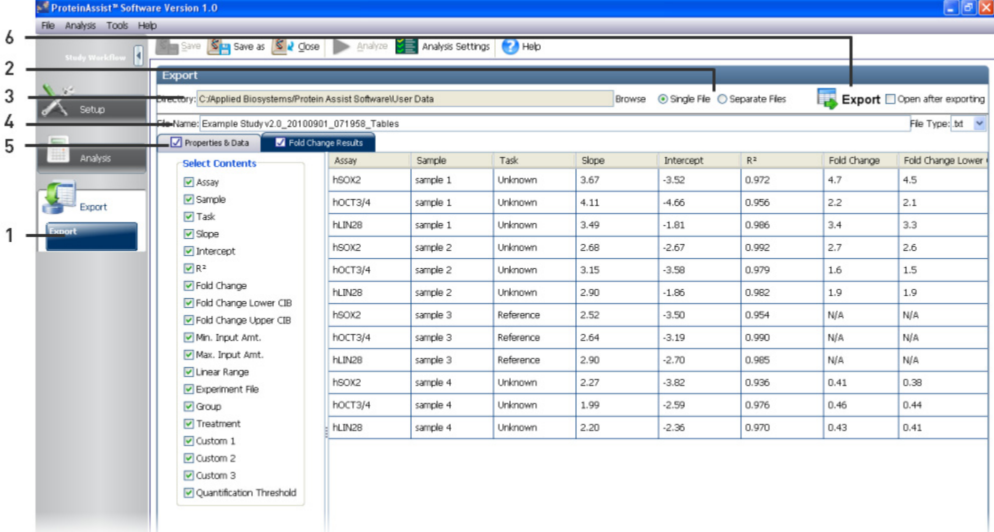
a. Under the Properties & Data tab, check the data to be exported.

b. Under the Fold Change Results tab, check the data to be exported.

6. Check/uncheck **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 a *.csv file in your default spreadsheet software.

Figure 38 Exporting study results



Open and view the exported files

1. Right-click the exported file, then select **Open With** ▸ **Microsoft Office Excel** to view the contents as a spreadsheet.

2. Use the AutoFit function in Excel software 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

Observation	Possible cause	Recommended action
Hundreds of sample names display in the Samples list.	The PCR instrument plate file has one sample name assigned per well.	**1.** Assign one sample name to all the wells in each dilution series. Refer to "Assign well properties" starting on page 30. **2.** Delete the unused sample names: In **Setup** ▸ **Samples**, select the sample names to be deleted (ctrl-click or shift-click), then click **Remove**.
Custom field names or field values are truncated. The software alerts you that only visible text is stored.	ProteinAssist™ Software truncates field values that exceed the maximum length allowed.	Adjust the entry so that all text is visible.
Atypical data is displayed in wells or samples.	Contaminated wells or samples.	Repeat the TaqMan® Protein Assays with fresh reagents. Refer to *TaqMan® Protein Assays Sample Prep and Assay Protocol*.
	Instrument is not correctly calibrated.	Make sure your instruments are appropriately maintained and calibrated. Refer to the user guide for your instrument.
	Incorrectly labeled wells or samples.	Correct the well or sample property.
You are unable to edit well properties using the Well Explorer tool.	More than one well is selected. (The Well Explorer tool can only be used to edit one well's properties.)	Use the Well Editor to edit properties for multiple wells. See page 31.
	You are trying to change an uneditable property. (The Well Explorer tool cannot be used to change the well location, C_T value, or Task.)	Use the Well Editor or Tasks list to change a well's Task assignment. The C_T value or well location cannot be changed.
Inadvertent change to well property.	Wells were selected with the Dilution tool instead of the selection tool.	Ensure that the correct cursor is displayed when selecting wells. Refer to Table 9 on page 28.
	Incorrect well property for an NPC sample was assigned after NPC task or sample assignment.	When the NPC sample or task is assigned, the input quantity is set to 0 and other well properties are cleared. Assign the NPC task or sample to a well after all other properties have been assigned, so that the well properties are reset appropriately.

Observation	Possible cause	Recommended action
The study fails to analyze, and a field error icon appears in the plate layout grid or in the experiment files list.	Error(s) in experiment file(s) setup.	Hover the pointer over the field error icon for information to correct the setup. Below are some common setup corrections. See also "Analysis rules" on page 30. • Make sure that the correct Reference Use is selected in the Edit Study Property screen: – **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. • Make sure that each plate has at least one no-protein control (NPC) assigned per assay. • Make sure that each assay-sample combination is assigned a task, either "Unknown," "Reference," or "NPC." • Make sure that each assay-sample combination with a task assignment of "Unknown" is unique across the study. • Make sure that every assay has at least one unknown sample.
The linear range column on the sample table has a value "Unknown."	The slope of the regression line within the linear range is <0.1.	If the slope is <0.1 because of low concentration of the target protein, prepare new lysates with a higher concentration of cells, and repeat the TaqMan® Protein Assays. See also "Software rules for the fold change algorithm" on page 64.
Fold change for a sample is "Undetermined."	All the values for ΔC_T for that sample are <0.5.	If ΔC_T values are <0.5 because of low concentration of the target protein, prepare new lysates with a higher concentration of cells, and repeat the TaqMan® Protein Assays.
	The fold change confidence interval is infinite.	• Review the linear range. • Omit outliers from data analysis. • See also "Software rules for the fold change algorithm" on page 64.
Software fails to open, and the following error message displays: "JVM terminated. Exit code=1..."	Intermittent Java Runtime error.	Click **OK**, then open the software again.

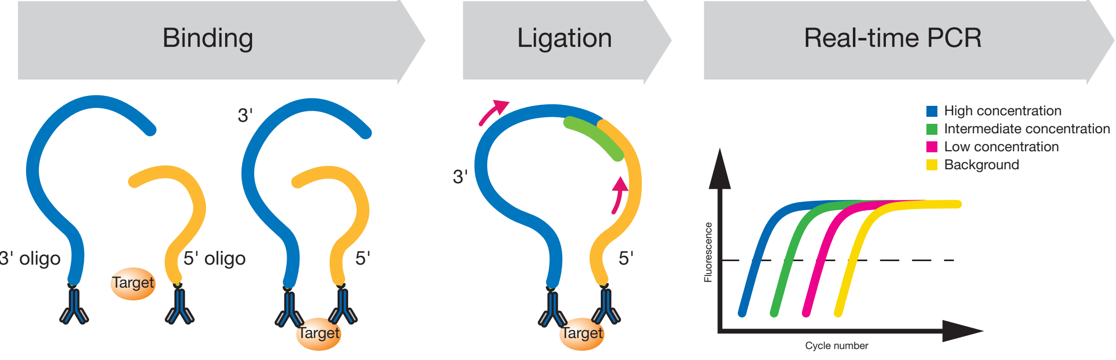
Supplemental information

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 39).

In the TaqMan Protein Assay procedure, 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 Assays** Chemistry Guide* (PN 4405780) and the *TaqMan® **Protein Assays** Sample Prep and Assay Protocol* (PN4449283).

Figure 39 TaqMan® Protein Assays: proximity ligation with real-time PCR



Key features of TaqMan® Protein Assay data analysis

The C_T 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.

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 (cell lysate) is assayed, 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 example, in 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.

"Fold change algorithm" on page 62 gives a detailed description of the algorithm.

Linear range algorithm

ProteinAssist Software automatically selects a set of data for each sample that follows a linear relationship between log(input quantity) and ΔC_T. The zone encompassing the upper and lower boundaries of the data set is called the linear range. The slope and intercept of the regression line through points encompassed by the linear range interval are key parameters in the fold change algorithm (see "Fold change algorithm" following).

In general, a plot of TaqMan Protein Assay ΔC_T values vs log(input quantity) for a dilution series forms a sigmoidal curve. The appropriate linear region of this curve to include for the purposes of estimating fold change is the transition region, the interval over which the sigmoid moves from low ΔC_T to high ΔC_T values.

Figure 31 on page 49 illustrates an example of an adjustment to the linear range so that it better reflects the transition region.

Fold change algorithm

The fold-change estimate is intended to estimate the amount of a target protein contained in a target sample relative to that contained in a reference sample. It is applicable to situations where the amount of target protein per cell cannot be determined but the exact number of cells or the total protein concentration 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 slopes 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:

$$F = b^{(B_{unk} - Q_T)/A_{unk} - (B_{ref} - Q_T)/A_{ref}}$$

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:

- When cell count is used to normalize the data, it is assumed that 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; that is, the distribution of per-cell protein content is assumed monomodal over the population of cells analyzed.
- 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.
- The value of Q_T is appropriate for the unknown and reference samples.
- 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.

Fold change confidence interval

The 95 % confidence interval for the fold change estimate is obtained by combining the standard confidence intervals (95 %) for the linear regressions performed on the reference and unknown data within their respective linear ranges.

The nature of the confidence interval calculation is such that, for studies with multiple experiment files and one reference sample (Reference Use is Per Study), the confidence interval estimate will be more accurate if the reference sample replicates are spread out across the plates.

Setting the Quantification Threshold

If the slopes of the reference and unknown samples are equal in the linear range (that is, the curves are parallel in this region), then the value of Q_T, the Quantification Threshold, has no impact because it drops out of the fold change calculation above.

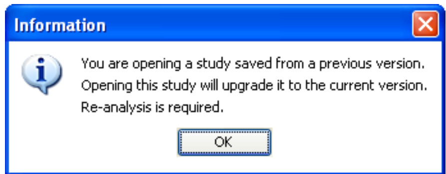
If the slopes for the samples are not equal, the most accurate fold change calculations are achieved by determining the appropriate value of the Quantification Threshold empirically, as described in "Calibrate the Quantification Threshold" on page 67. Use the default Quantification Threshold setting for experiments where the Quantification Threshold has not been empirically determined.

Software rules for the fold change algorithm

If...	Then...
The quantification threshold line does not intersect the 95 % confidence bounds of the regression line for either the reference sample, the unknown sample, or both. (This can happen for data with a high variance or a low number of data points.)	The fold change confidence interval upper and lower boundary values display "Undetermined."
Valid confidence intervals are ***not*** generated.	The fold change value displays "Undetermined."
The majority of the points in each dilution group are <0.5 ΔC_T.	The fold change value displays "Undetermined."
The majority of the points in each dilution group are <0.5 ΔC_T ***but*** at least one dilution point has a majority of replicates >0.5 ΔC_T.	The slope, intercept, fold change value, and confidence interval boundaries are calculated and displayed.
The determined slope for a given sample is <0.1.	• The slope and intercept display "N/A." • The fold change value and confidence interval boundaries display "Undetermined." • The Linear Range column (**Analysis ▸ Linear Range ▸** sample table) displays "Unknown".
The linear range is set manually.	• The 0.1 slope limit does ***not*** apply. • The 0.5 ΔC_T rules ***do*** apply.

Upgrade conversions of pre-release data

The first time you open a study from the pre-release version of ProteinAssist Software, you receive a prompt to re-analyze the study. Click **OK**.



When the pre-release data is re-analyzed, the following conversions make the pre-release data compatible with the upgraded software.

- The Linear Range Detection sensitivity is set to 20 %.

  To see the setting, go to **Study Analysis toolbar ▸ Analysis Settings**.
- The contents of the pre-release time field are moved to an unused custom field. (Refer also to the entry for custom fields in Table 5 on page 20.)
- Analysis results are cleared.
- Manual linear range settings are retained.
- Samples with task assignments of "NPC" are assigned "NPC" as sample name.
- Samples with names of "NPC" are assigned the task "NPC."

Related documentation

Title (Part Number)	Description
TaqMan® Protein Assays Sample Prep and Assay Protocol (PN 4449283)	Procedures for sample prep and relative quantification experiments with TaqMan® Protein Assays reagents.
TaqMan® Protein Assays Chemistry Guide (PN 4405780)	Background and supplemental information on TaqMan® Protein Assay technology and targeted proteins.

Obtaining support

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- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Supplemental procedures

Calibrate the Quantification Threshold

The accuracy of fold change estimates can be improved by calibrating the Quantification Threshold. Ideally, the calibration is performed for each distinct assay-sample type combination. The following procedure describes how to perform the calibration.

1. Prepare positive and negative control sample lysates for the TaqMan® Protein Assay target of interest, as described in the *TaqMan® **Protein Assays** Sample Prep and Assay Protocol*.
2. Mix positive and negative control sample lysates to generate a set of samples with known relative amounts of target.
3. Prepare a dilution series from each mixed lysate, and perform TaqMan Protein Assays with the assay of interest, as described in the *TaqMan® **Protein Assays** Sample Prep and Assay Protocol*.
4. Set up the experiment files in ProteinAssist™ Software.
5. In **Analysis ▸ Linear** Range, adjust the Quantification Threshold systematically and compare the observed fold change results to the theoretical values. See Figure 40 on page 68.

   To adjust the Quantification Threshold setting:

   a. Enter the threshold value in the ΔC_T Plot toolbar.

      or

      Click-drag the horizontal dashed line and move it up or down.

   b. Click **Analyze** in the toolbar to recalculate the plot with the new Quantification Threshold.

   The Quantification Threshold adjustment applies to all samples for that assay, within the study.

The Quantification Threshold setting that generates fold change data that best reflects the theoretical values should then be used for unknown samples.

In the example shown in Table 17 on page 68 and Figure 40 on page 68, NTERA2 (positive) and Raji (negative) control lysates were mixed to yield a series of lysates that were 100%, 50%, 25%, and 10% NTERA2. These mixed lysates were used for hLIN28 TaqMan Protein Assays. The 100% NTERA2 sample served as the reference.

Table 17 shows the observed fold change results at different Quantification Threshold settings. In this example, Quantification Threshold settings of 2.5 or 3.0 resulted in fold change values calculated by the software that were closest to the theoretical values based on the proportion of NTERA2 lysate in each sample.

Figure 40 shows the dilution series for the samples at a Quantification Threshold setting of 2.5.

Figure 40 Calibrating the Quantitation Threshold

Table 17 Observed fold change results at various Quantification Threshold settings

Sample	Theoretical Fold Change	Observed Fold Change at Quantification Threshold Setting:		
		2.0	2.5	3.0
100 %	N/A	N/A	N/A	N/A
50 %	0.50	0.57	0.52	0.48
25 %	0.25	0.29	0.27	0.25
10 %	0.10	0.12	0.10	0.083

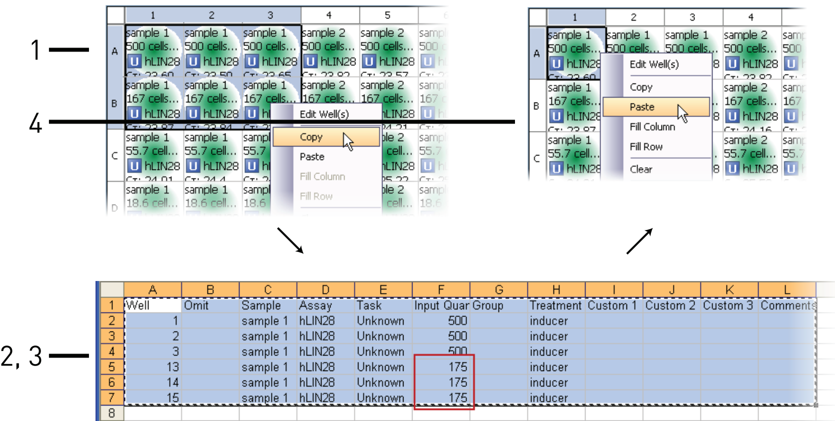
Use Microsoft® Excel® software to modify well properties

Users familiar with Microsoft Excel spreadsheet software may find it useful to modify certain well properties in an Excel spreadsheet.

1. Select one or more wells and right-click to access the well menu. Select **Copy**.
2. Open a blank Excel worksheet and use the Paste function to copy the well properties into the worksheet.
3. After making the desired edits in Excel, copy all rows and columns with data or annotation, including column headers.

   Do not modify the header cells in Excel.

4. Right-click the *top-left* well of the selected group in the Plate Layout grid, and select **Paste** to paste the edits for all the selected wells.



Glossary

ΔC_T	The C_T value for a sample after correction for the NPC (no protein control) C_T (background ligation). ΔC_T = average NPC C_T – sample C_T.
experiment file	An experiment file contains TaqMan® Protein Assays data from a single reaction plate.
Export	The Export tools export data outside the application workspace for downstream analysis. You can export both plate setup data and analysis results.
Import	The Import tool imports experiment files from your real-time PCR instrument software into ProteinAssist™ Software.
No Protein Control (NPC)	TaqMan® Protein Assay samples without cell lysate. The NPC samples measure the background ligation of TaqMan Protein Assays.
plate file	In the real-time PCR instrument software, a file that contains real-time PCR data from a single reaction plate.
properties	Data assigned to wells that are used in ProteinAssist™ Software analysis.
reference	Relative quantification (fold change) of the TaqMan® Protein Assay targets in the unknown samples is calculated relative to the reference sample. For example, in a time course study, the time-zero sample could be the reference.
task	The role of the sample in the data analysis: NPC, Reference, or Unknown.
Transfer out Study	The Transfer out Study tool saves study data outside of the application workspace as independent files, each with a .las file extension, for sharing or backup purposes. These files are still compatible with ProteinAssist™ Software, using the Transfer in Study tool.
Unknown	Unknown samples are the test samples for which the relative quantification of the TaqMan® Protein Assay targets is calculated.
*.csv	Comma-separated value file.
*.las	A study file that has been saved outside the application workspace using the Transfer out Study tool.
*.lpt	A template file that stores plate setup properties that are used frequently.
*.txt	Tab-delimited text file.

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applied biosystems™
by life technologies™

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone 760.603.7200
www.lifetechnologies.com

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