appliedbiosystems

GlycanAssure™ Data Analysis Software v2.0

USER GUIDE

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Revision C

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision history: Pub. No. 100036373

Revision	Date	Description
C	26 February 2018	Revision for v2.0 software. Add SAE functionality, auto alignment, and manual multi-peak alignment.
B	20 March 2017	Revision for v1.1 and v1.1.1software. Update: screen shots, search, import samples, analysis status, analysis method parameters, view results tables, view electropherograms, adjust delimiters and baseline. Add new information for create and manage bins, manage resources. Add new information for normalization and align peaks.
A	15 February 2016	New document for v1.0 software.

Contents

1 Get started

GlycanAssure™ Data Analysis Software

The GlycanAssure™ Data Analysis Software is a glycan analysis software solution.

The software uses a database host to store projects and settings.

System overview

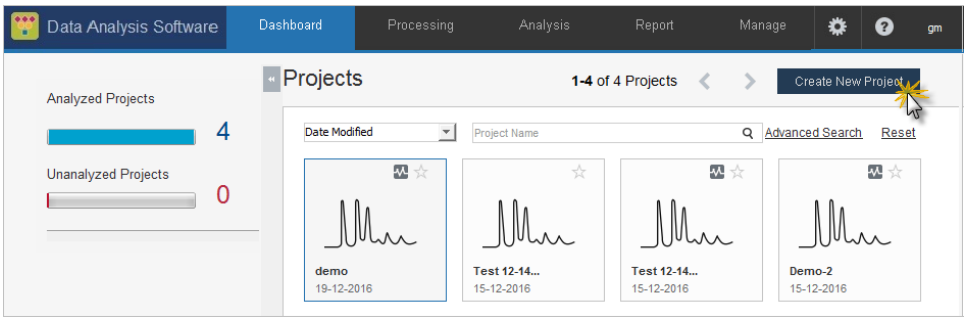
The GlycanAssure™ System is an integrated glycan analysis platform with three components:

1. **GlycanAssure™ Kits**—Provide reagents for sample preparation (to release, purify, and label glycans) and for capillary electrophoresis.
2. **Applied Biosystems™ 3500/3500xL Genetic Analyzer for Protein Quality Analysis** (POP-7™ polymer, 50-cm capillary array) (described in Pub. No. 100036372)—Runs GlycanAssure™ Data Acquisition Software to collect data for samples prepared with the GlycanAssure™ Kits.
3. **GlycanAssure™ Data Analysis Software** (described in Pub. No. 100036373)—Processes and analyzes glycan data and includes data trending and profile matching features.

Workflow

1 Dashboard

Create a project



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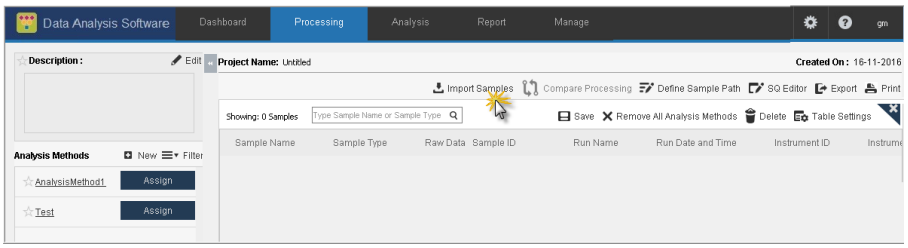
2 Processing

Import samples



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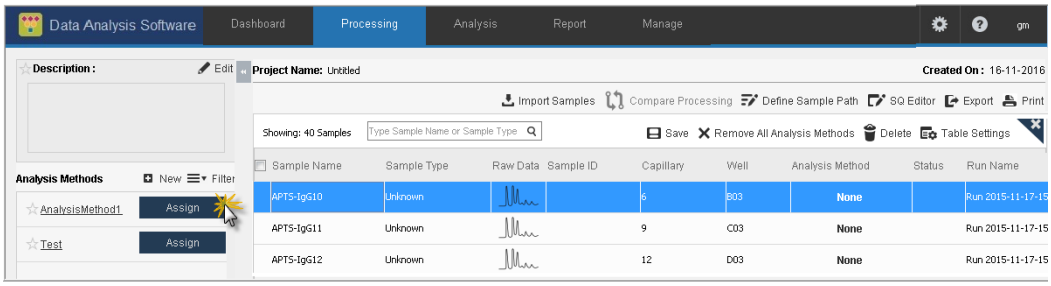
Assign analysis method



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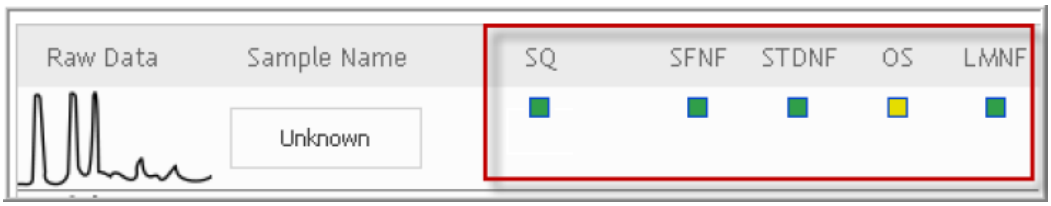
Review the Sizing Quality (SQ) and sample quality flags



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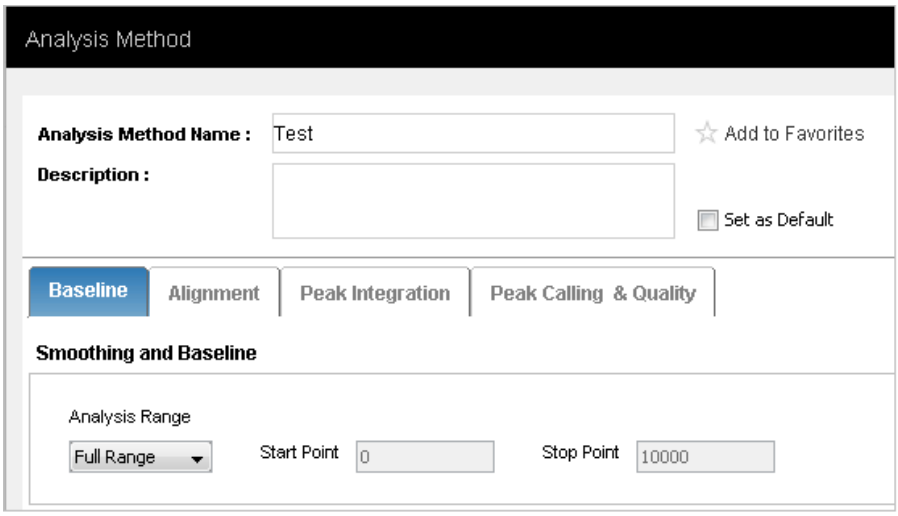
(Optional) Create or modify analysis method



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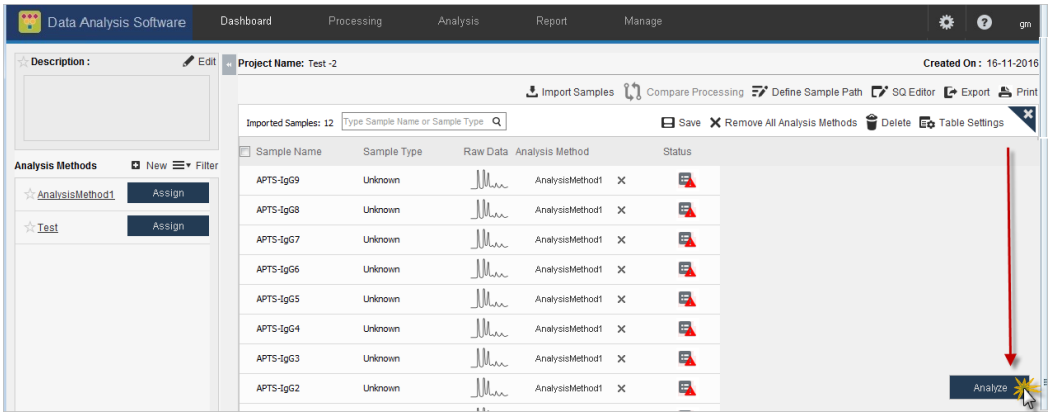
Analyze samples



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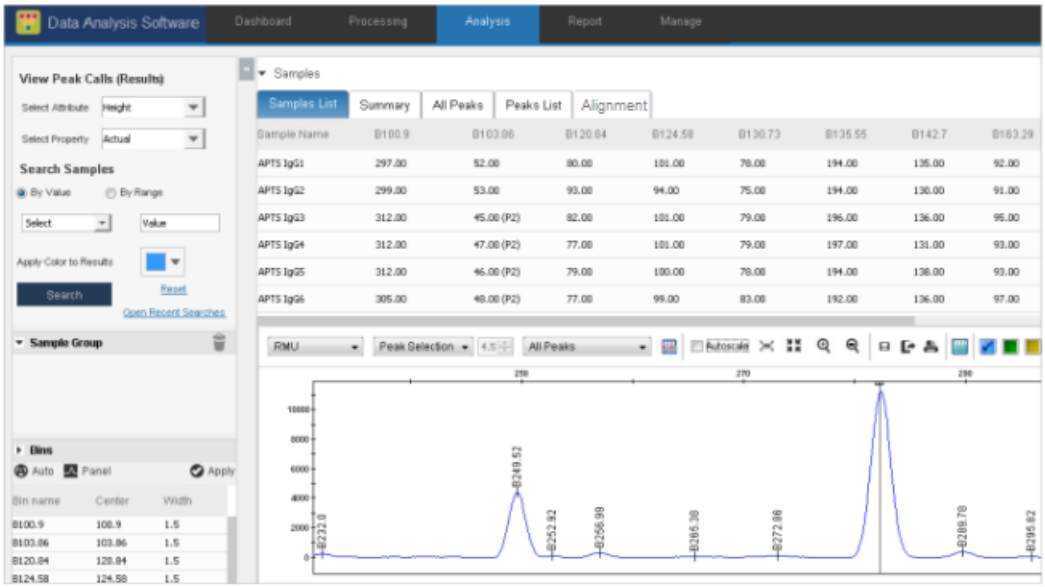
3 Analysis

Review analysis results

(Optional) Adjust peak delimiters, baseline, and bins



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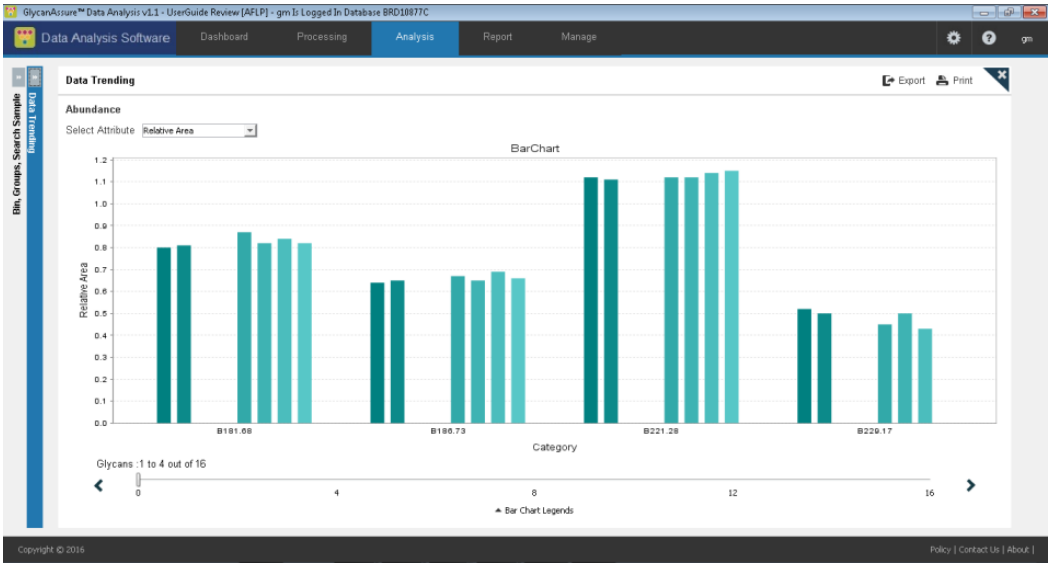
Review data trending



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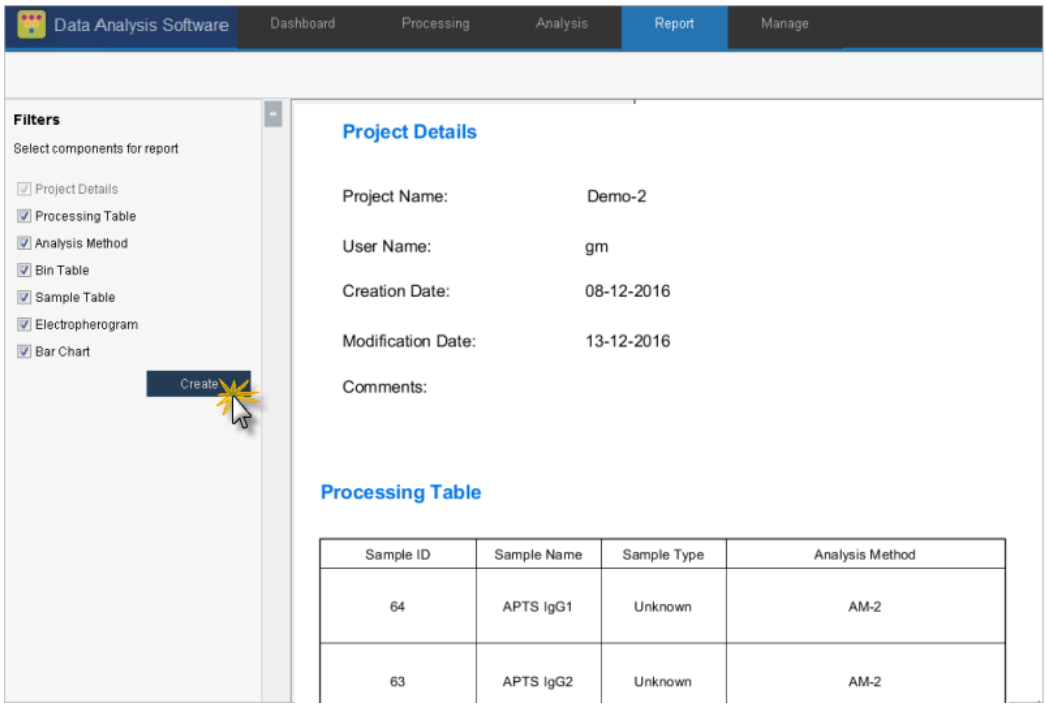
4 Report

Review reports



Set preferences

1. In the top right of the toolbar, click  .
2. Specify settings as needed, then click **Apply**.

Preference	Setting	Description
Dashboard	Show *number of* projects	Number of projects per page
	Refresh Run Count	For Autoanalysis. Not available in this release.
	Define Run Paths	For Autoanalysis. Not available in this release.
Processing	Save project	Sets automatic saving and interval.
	Show Analysis Methods	Determines the projects listed: All, Favorite, or Default.
	Show quality alerts	Determines whether flags are shown as colored indicators or numbers.
Analysis	Show quality alerts	Determines whether flags are shown as colored indicators or numbers.
	Data trending default	Determines if the trending default is Area, Relative Area, Height, Relative Height, or Retention Time.
Reports	Logo	Image (.jpg) to include in the report header.
	Header and footer text	Text to include in the report header and footer.
Date format	Region	US or UK (selection determines options displayed in Format list)
	Format	Date format

2 Work with projects

Create, open, or save a project

In the **Dashboard** tab:

- **Create** – Click **Create New Project**
- **Open** – Double-click a project
- **Save** – Click 

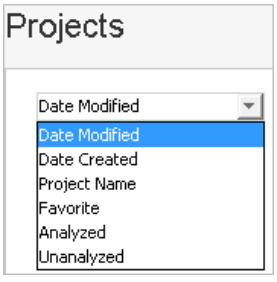
In the **Manage** tab:

- **Open** – Double-click a project
- **Save As** – Click 

Search for a project

You can do any of the following to search for projects:

- In the **Dashboard**, select a search criterion.



- In the **Dashboard**, click **Advanced Search**, select a search criterion, then set search parameters.

- In the **Manage** tab, select a criterion (name, date created or modified, user name), then click .

Import samples

1. In the **Processing** tab, click **Import Samples**.
2. Select the files or samples, then click **Import to Project**.

Analyze samples

In the **Processing** tab:

1. Select one or more samples.
2. Assign an analysis method (see "View, assign, or remove an analysis method" on page 15).
3. Click **Analyze**.

View a report

View a report from the **Report** tab by clicking **Create**.

Analysis status

Icon	Description
No icon	Not analyzed
	Analyzed
	• Analysis method was edited, removed from a sample, or deleted from the software (Manage>Analysis Method) • Size standard definition was edited or deleted from the software (Manage>Size Standard) • Samples are deleted from the project • Sample .fsa files are moved from their original location during analysis (using Windows™ File Explorer)

Processing flags

Table 1 Processing flags

Flag	Description	Status
SFNF	Sample File Not Found	Sample file is in the original location from which it was added to the project. Sample file is not in the original location from which it was added to the project.
STDNF	Size Standard Not Found	Size standard definition is specified in analysis method. Size standard definition is not specified in analysis method.
SQ	Sizing Quality	SQ value is in the Pass range SQ value is between the Pass range and the Low Quality range SQ value is In the Fail range
OS	Off Scale	No fluorescence signals within the analysis range exceed the signal threshold of the instrument One or more fluorescence signals within the analysis range exceed the signal threshold of the instrument
SQI	Sizing Quality Invalid	Override SQ applied
LMNF	Landmark Not Found	Number of landmark dye peaks detected is ≥ the number of peaks required by the software Number of landmark dye peaks detected is < the number of peaks required by the software No landmark dye peaks are detected

3 Work with analysis methods

View, assign, or remove an analysis method

In the **Processing** tab:

- To view an analysis method, click an analysis method name in the left pane.
- To assign an analysis method, you can:
  - Select individual samples, then click **Assign** next to the method to assign
  - Select **Select All Samples** from the drop-down list above the **Processing** table, then select the method to assign

  Note: Only one analysis method is allowed in a project to allow the same bins to be used by all samples in the project.
- To remove an analysis method, you can:
  - Click **Remove All Analysis Methods** to remove the analysis method from all samples
  - Click **x** next to the method to remove
  - Right-click a row in the table, select **Remove Analysis Method**, then select the method to assign

After adding or removing an analysis method, click **Analyze** to apply the changes. Samples that do not have an analysis method assigned are not analyzed.

Filter analysis methods

- In the **Processing** tab, click ☰▾, then select **All**, **Favorite**, or Default analysis **methods**.
- Set the **Processing** tab preference to display All, Favorite, or Default analysis methods. You can override the preference setting by clicking ☰▾.

Analysis method parameters

Use the default settings as a starting point and adjust for your data as needed.

Table 2 Analysis method parameters

	Parameter	Description
General	**Analysis Method Name** **Description**	Unique name and optional description.
	Add to Favorites	Click to tag as a favorite. You can set preferences to show only favorite analysis methods in the **Analysis** tab.
	Set as default	Select the checkbox to specify as the default method. You can set preferences to show only the default analysis methods in the **Analysis** tab. You cannot delete or modify a method specified as the default method.
Baseline tab	**Analysis Range**	Specify the range (in data points) to analyze: • **Full Range** to analyze the entire scan region as collected by the capillary electrophoresis instrument, including the Landmark Red peak. • **Partial Range** to analyze only data points within a specified range. Enter Start Point in data points after the primer peak and before the first required size standard peak. Enter a Stop Point after the last required size standard fragment. Start and Stop points may vary from instrument to instrument and platform to platform. Display raw data to determine the appropriate analysis range. Data points outside the specified range are ignored. Ensure the **Analysis Range** contains all size standard peaks.
	Smoothing	Select a smoothing level (0 to 8) to smooth the outline of peaks and reduce the number of false peaks detected: • Use 0 if the data display sharp, narrow peaks of interest. • Use a low number to provide the best results for typical data. Light smoothing slightly reduces peak height. • Use a high number for data with broad peaks of interest. Heavy smoothing can significantly reduce peak height.
	Baseline Window	Specify a window to adjust the baseline signals of all detected dye colors to the same level for an improved comparison of relative signal intensity. Note the following: • A small baseline window relative to the width of a cluster, or grouping of peaks spatially close to each other, can result in shorter peak heights. • Larger baseline windows relative to the peaks being detected can create an elevated baseline, resulting in peaks that are elevated or not resolved to the baseline.

	Parameter	Description
Baseline tab	**Normalization**	• Size standard (described below) • Average signal of the data in a sample file • Maximum signal of the data in a sample file **Size standard normalization** The Data Acquisition Software provides a normalization option that is enabled through the size standard selection. It uses the GeneScan™ 600 LIZ™ Size Standard to obtain consistent lot-to-lot peak heights. It provides more consistent results between data from different instruments, injections, and capillaries. The software calculates a normalization factor based on a threshold setting. For each injection, the normalization factor is used as a multiplier to adjust the peak height of the sample peaks relative to the GS600 LIZ™ size standard peaks. There are minimum and maximum limits to the normalization factor, so if the size standard peak heights are abnormally high or low, the normalization will be limited. The normalization factor is saved in the sample data file. When you import a normalized data file in to a project, you can remove and apply the normalization factor by deselecting and selecting the Enable Size Standard Normalization check box.
Alignment tab	**Size Calling Method**	Select the method to determine the molecular length of unknown fragments. • **Second Order Least Squares** - Uses regression analysis to build a best-fit size calling curve. • **Cubic Spline Interpolation** - Forces the sizing curve through all the known points of the selected size standard. • **Global Southern Method** - Compensates for standard fragments with anomalous electrophoretic mobility (similar to least squares methods). • **Third Order Least Squares** - Uses regression analysis to build a best-fit size calling curve. • **Local Southern** - (default) Determines the fragment sizes using the reciprocal relationship between fragment length and electrophoretic mobility.
	Size Standard	Select a the size standard definition for the size standard used for the samples.

	Parameter	Description
Alignment tab	**Auto Alignment**	Select the checkbox to enable automated alignment of the sample peaks with reference peaks. The following peak properties for each reference standard determine the automated alignment of sample peaks with the reference peaks: • Peak positions • Offset for each peak • Order of polynomial regression • Dye color **Note:** Auto alignment is disabled by default. Select the checkbox to enable auto alignment.
Peak Integration tab	**Peak Start and Stop**	• **Peak Start** - The peak starts when the first derivative (slope of the tangent) in the beginning of the peak signal before the inflection point becomes equal to or exceeds the Peak Start value. This threshold is set to 0 by default, which means that the peak will normally start at the leftmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak start point toward its center. The value entered must be non-negative. • **Peak Stop** - The peak ends when the first derivative (slope of the tangent) in the end of the peak signal after the inflection point becomes equal to or exceeds the Peak End value. This value is set to 0 by default, which means that the peak will normally end at the rightmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak end point toward its center. The value entered in this field must be non-positive.
	Minimum Peak Half Width	Specify the smallest half peak width at full height for peak detection. The range is 2 to 99 data points.
	Polynomial Degree	**Polynomial Degree** cannot be greater than **Peak Window Size**. Adjust to affect the sensitivity of peak detection. You can adjust this parameter to detect a single RMU difference while minimizing the detection of shoulder effects and/or noise. The peak detector calculates the first derivative of a polynomial curve fitted to the data within a window that is centered on each data point in the analysis range. Using curves with larger polynomial degree values allows the curve to more closely approximate the signal and, therefore, captures more of the peak structure in the electropherogram.

	Parameter	Description
Peak Integration tab	**Peak Window Size**	Enter a window width in data points for peak detection sensitivity. If more than one peak apex is within the window, all are labeled as a single peak. Note the following: • The maximum value is the number of data points between peaks. • The **Peak Window Size** setting is limited to odd numbers. To increase peak detection sensitivity: Increase **Polynomial Degree**, decrease **Peak Window** Size. To decrease peak detection sensitivity: Decrease **Polynomial Degree**, increase **Peak Window Size**.
	Absolute Height (Peak Amplitude Threshold)	Specify the threshold (RMU) for peak detection for each dye color. Peaks ≥ the threshold are detected. For example, if you use the default values of 50, peaks with heights equal to or greater than 50 are detected. Peaks with heights below 50 are still displayed in the electropherogram plots but are not detected or labeled.
Peak Calling and Quality tab	**Peak Window (for BD flag)** **Max. Peak Width**	Max Peak Width Threshold to identify broad peaks (BD).
	Pull-up Peak (for SPU flag)	The pull-up ratio or scan for a peak to be called a pull-up peak. Pull-up occurs when the peak height of the called peak is less than X % of the larger peak that is within ±1 data point.
	Sizing Quality Range (SQ)	Pass, Check, and Fail ranges for SQ flag.

4 Analyze and review results

Analyze samples

In the **Processing** tab:

1. Select one or more samples.
2. Assign an analysis method (see "View, assign, or remove an analysis method" on page 15).
3. Click **Analyze**.

Align peaks automatically

When auto alignment is enabled, the software automatically determines the alignment of sample peaks with the reference peaks.

Enable auto alignment in the analysis method assigned to your samples:

1. In the **Processing** tab, click the analysis method name in the left pane.
2. Click the **Alignment** tab.
3. Select the **Auto Alignment** checkbox, then click **Save**.
4. Click **Analyze**.

The software calculates the new RMU based on the number of peaks specified and detected.

Note: The software does not change the peak area or height attributes after alignment.

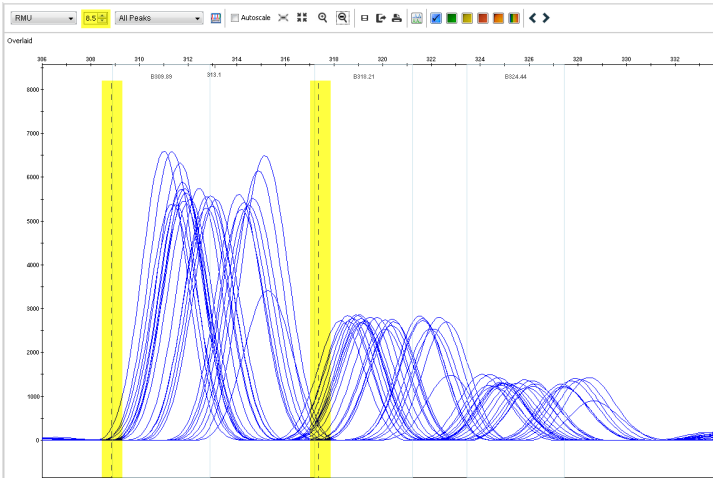
If you are not satisfied with the results of the auto alignment, you can perform manual alignment (see page 21).

(Optional) Align peaks manually

If the RMU for a given peak varies between samples, the peak may be assigned to an incorrect bin. To align the peaks across samples, you can select one or more peaks manually.

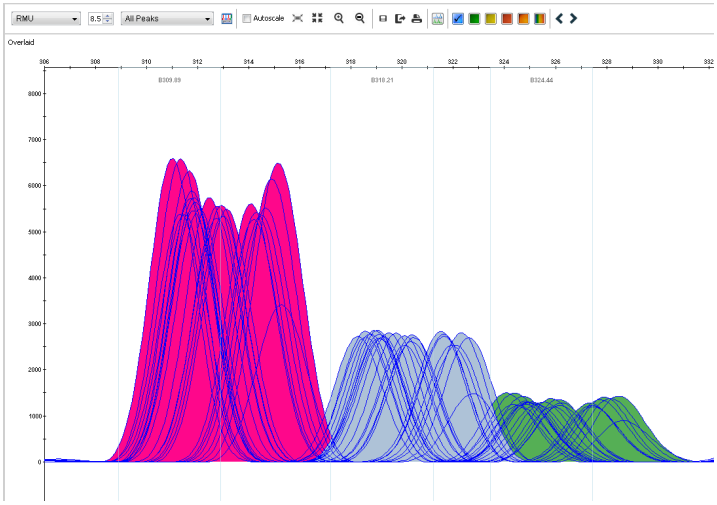
In the **Analysis** tab:

1. Click the **Alignment** tab.
2. If individual traces are displayed, click  to overlay traces.
3. Zoom-in on the peaks you want to align.
4. Set the window width so that the window edges encompass all of the peaks you want to align.

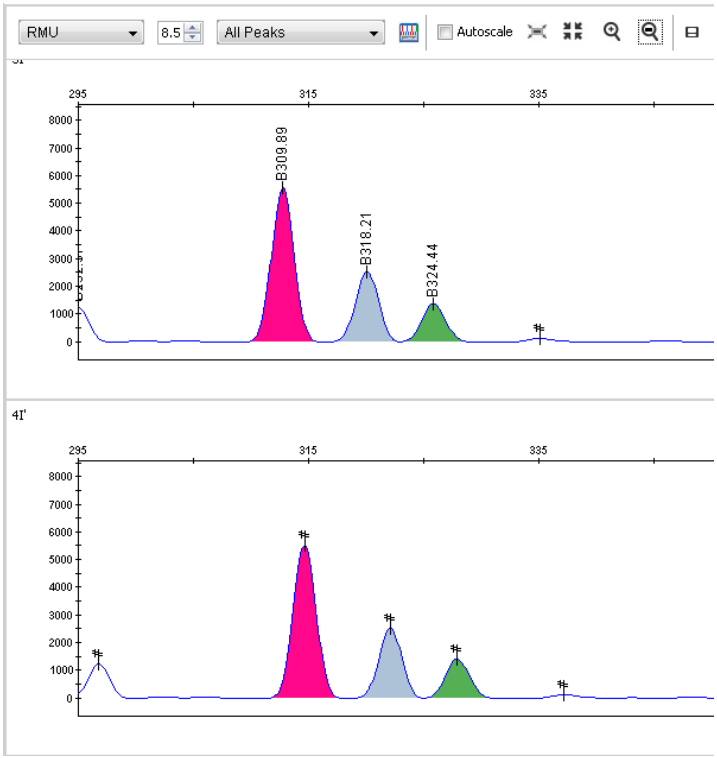


5. When the window is properly positioned, click the trace to select all peaks within the window edges.
   The selected peaks are assigned the same color.

6. Align peaks in a different region of the trace by repeating step 3 through step 5.

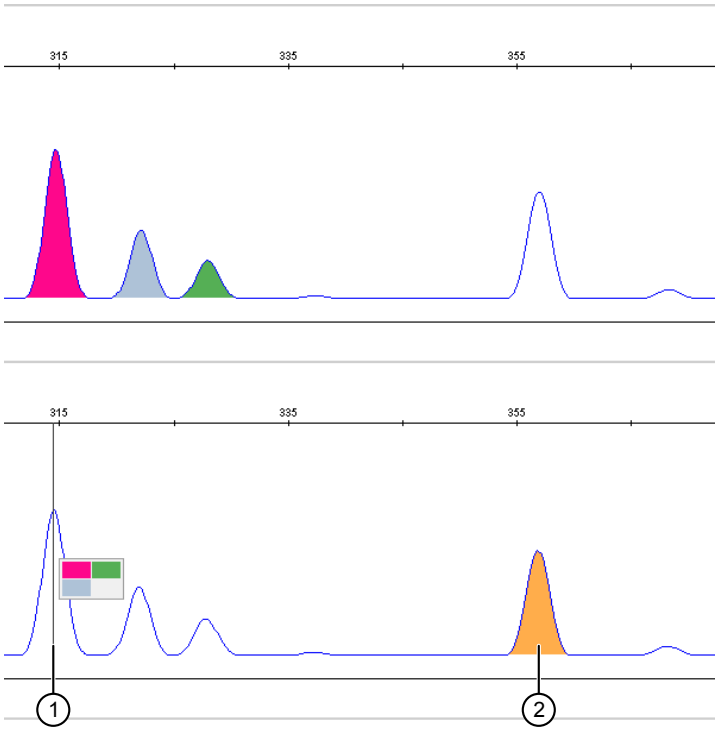


7. Click  to display individual traces, then scroll through each individual trace to confirm that the peaks are correctly selected.



8. If a peak is unassigned or assigned incorrectly:
   a. If needed, click the peak to remove the incorrect color assignment.

b. Right-click the peak to select a color from currently assigned colors, or click the peak to assign a new color.



① Right-click to select a color from currently assigned colors
② Click to assign a new color

9. Click **Apply**, then click **Yes** to confirm.

Note: The software does not change the peak area or height attributes after alignment.

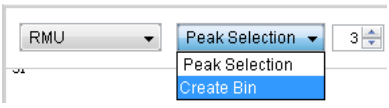
Click **Reset** to remove the offsets and alignment. Click **Cancel** to remove the selection.

Create and manage bins

Create new bins manually

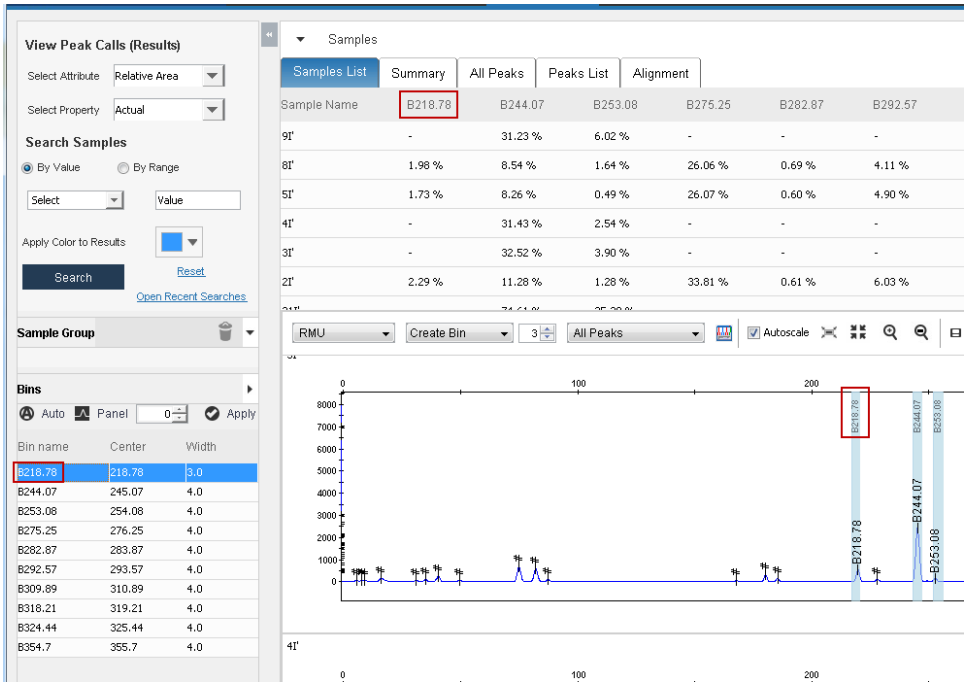
In the **Analysis** tab:

1. Select **RMU**, select **Create Bin**, then select the bin width.



2. Click a location in the plot to add a bin.

   Bins are displayed as blue bars in the electropherogram plot. Bin values are listed in the left pane.



3. Click **Apply** to save the result and add the bin to the **Samples List** and **Summary** table.

Create new bins with autobinning

The autobinning function creates bins based on:

- The peaks that are detected with the settings in the analysis method
- The bin width that you specify

In the **Analysis** tab:

1. Click **Auto** in the left pane.

2. Select the sample, range, and bin width to use to create bins, then click **Autobin**.
   The bins are displayed in the trace.

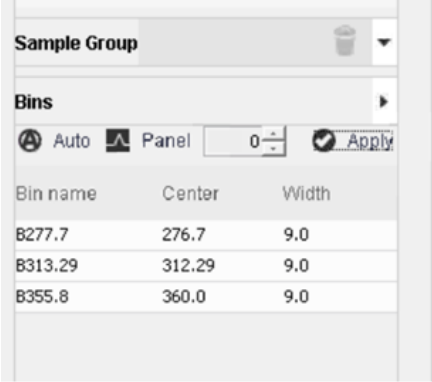
3. *(Optional)* If expected bins are not created for small peaks, click **Assign Empty Bins**, specify a peak amplitude threshold to detect smaller peaks, then click **Apply Second Pass**.
   The peaks are displayed in the trace with "P2" to indicate the peak amplitude threshold from the analysis method has been overridden.

   Note: To remove P2 peak labels, click **Assign Empty Bins**, then click **Remove Second Pass**.

Apply, save, or shift a panel of bins

In the **Analysis** tab:

- To apply a previously saved panel of bins, click Panel, select a panel, then click **Import**.
- To save a panel of bins for future use, click **Add to Library**.
- To shift a panel of bins left or right, change the values for one or more bins, or use the arrows to shift the panel (–10 through +10), then click **Apply**.

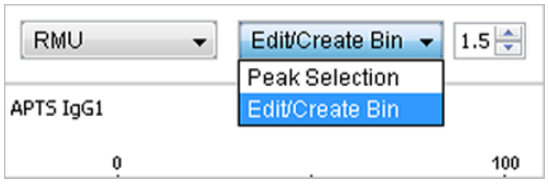


Note: Use the shift feature if the applied panel of bins is not properly assigned to the aligned peaks.

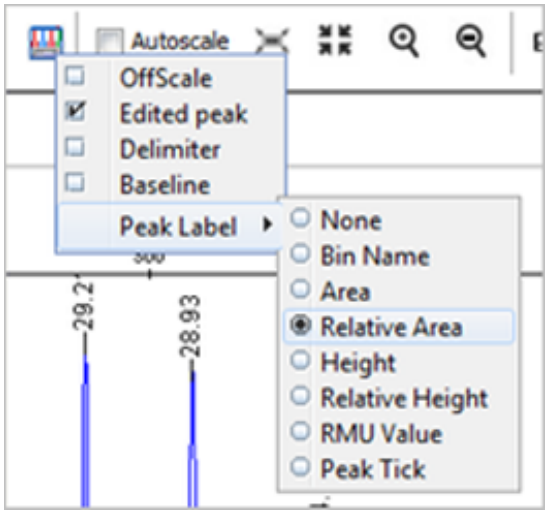
Display bin information in the plot

In the **Analysis** tab:

- To display bins in the plot, select **RMU**, select **Edit/Create Bin**.



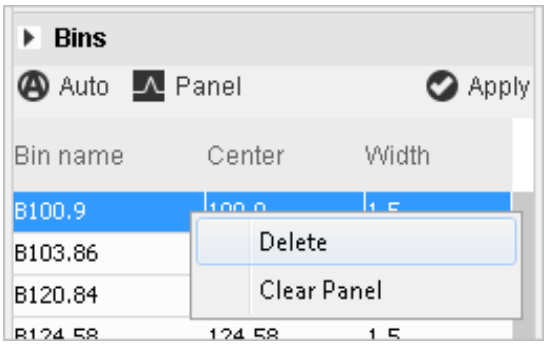
- To display bin names on the peaks, click , select **Peak Label**, then select **Bin Name**.



Edit, unassign, or delete bins

In the **Analysis** tab:

- To edit a bin, double-click a bin name, center, or width value in the left pane, change the value, then press **Enter**.
- To unassign a bin, right-click a peak in the plot, then select **Unassign bin** or **Unassign all bins**.
- To delete a bin, click a bin name in the left pane, right-click, then select **Delete**.



Remove P2 peak labels

A (P2) peak label is automatically assigned to peaks that are detected with a Peak Amplitude Threshold lower than the threshold in the analysis method (assigned during autobinning). For more information, see "Create new bins with autobinning" on page 24).

Click **Assign Empty Bins** in the left pane, then click **Remove Second Pass**.

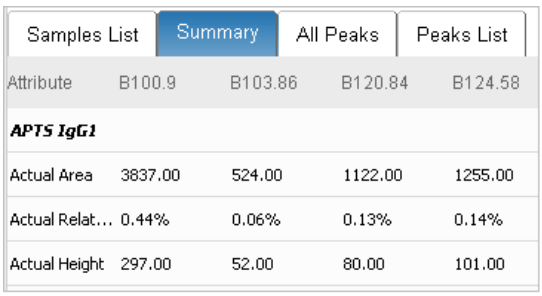
Examine results

View results tables

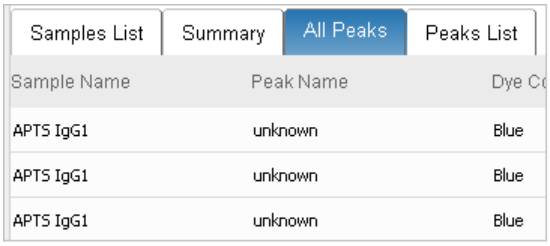
In the **Analysis** tab for an analyzed project:

1. Examine the **Samples list** tab for quality and bin information.

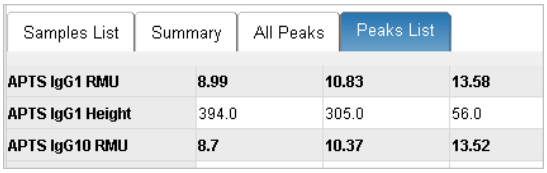
2. Examine the **Summary** tab for bin information on assigned peaks.



3. Examine the **All Peaks** tab for area, height, RMU, retention time, and datapoints on assigned and unassigned peaks.



4. Examine the **Peaks list** tab for a list of peaks that meet the **Peak Amplitude Threshold** in the analysis method and the **RMU** value for each peak.



Note: For information on the **Alignment** tab, see "Align peaks automatically" on page 20 and "(Optional) Align peaks manually" on page 21.

View electropherogram plots

To display electropherogram plots for an analyzed project, click the **Analysis** tab.

Do the following as needed.

- Assign bins (see "Create and manage bins" on page 23).
- View traces. The following plot settings are automatically applied: Blue dye, RMU, Autoscale on (adjusts the Y-Axis to the highest peak in zoomed area), Offscale off, Bin Name on, and Edited Peak indicator on.
  Peaks that have had peak start, peak end, or baseline modified are flagged with ★.
  Peaks that are detected with a Peak Amplitude Threshold lower than the threshold in the analysis method (assigned during autobinning) are flagged with (P2). For more information, see "Create new bins with autobinning" on page 24).
- Check for expected peaks by scrolling through all traces using the scroll bar on the far right of the pane, or clicking .
- View peaks assigned to bins and peaks not assigned to bins.
- Right-click a peak, then:
  - Assign a peak to a bin or remove a peak from a bin (unassign).
  - Lock the peak to prevent adjustment of peak start/end or baseline.
- Remove bins from analysis by right-clicking in the left pane, then selecting **Delete**.
- Search for peaks with specific attributes, for example, peaks with an area below a minimum setting you specify ("Search for peak calls (results)" on page 30).

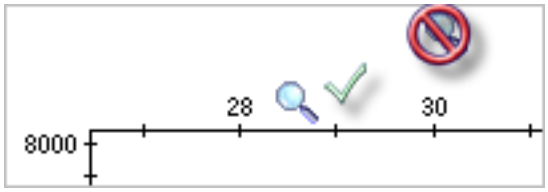
- Zoom (see "Zoom and unzoom" on page 28).
- Show offscale data by clicking , then selecting **Offscale**.
  Offscale peaks are flagged with pink bars.
- Adjust peak detection ("Adjust delimiters (peak start/end) and baseline" on page 29).
- Create sample groups for analysis and trending ("Create sample groups" on page 33).
- Change peak label settings ("Set the peak label to display" on page 37).

Zoom and unzoom

To zoom and unzoom on the x axis:

- **Zoom** –
  - Click *or*
  - Place the pointer *above* the plot until it turns into , then click-drag a box around the area *or*
  - Place the pointer *above* the plot until it turns into , right-click, select **Zoom To**, then enter the x-axis unit to zoom to
- **Unzoom** –
  - Click or *or*
  - Place the pointer *above* the plot until it turns into , right-click, then select **Full View** *or*
  - Place the pointer *above* the plot until it turns into , move it close to the top axis of the plot, then double-click

    Note: If you move too far away from the top axis of the plot, double-clicking does not unzoom.



To zoom and unzoom on the y axis:

- **Zoom** –
  - Place the pointer *to the left* of the plot until it turns into , then click-drag a box around the area *or*
  - Place the pointer *to the left* of the plot until it turns into , right-click, select **Zoom To**, then enter the y-axis unit to zoom to
- **Unzoom** –
  - Place the pointer *to the left* of the plot until it turns into , then double-click *or*
  - Place the pointer *to the left* the plot until it turns into , right-click, then select **Full View**

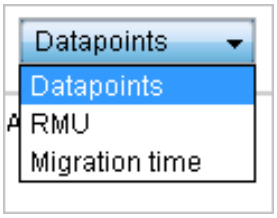
Adjust delimiters (peak start/end) and baseline

Before adjusting delimiters, assign bins (see "Create and manage bins" on page 23).

Locked peaks can not be edited. Right-click a peak, then select **Unlock**.

In the **Analysis** tab:

1. Select **Datapoints** from the plot toolbar.



2. Select **Edit Delimiters** or **Edit Baseline**.

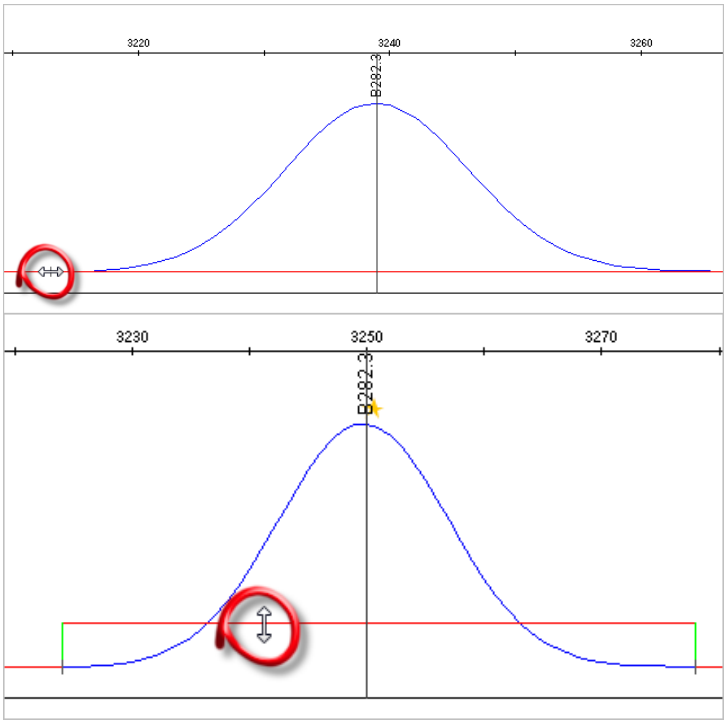
3. Click , then select **Delimiters** or **Baseline**.

4. Zoom on the x axis and y axis of an assigned peak until you can see peak start and end tick marks.

   To zoom, move the cursor to the left of or above an axis until it changes to , then drag around the area to zoom.

5. Click a start or end tick mark or the baseline. When the cursor changes to , click-drag the tick mark or baseline to a new location.

   Note: If you are editing the baseline and the cursor does not change to , zoom in on the y axis. You may need to zoom to <500 RFU.



6. Click to save the settings for the sample.

7. *(Optional)* Click , then select **Edited Peak** to flag the peaks with adjusted delimiters or baseline.

Search for peak calls (results)

You can specify new search parameters, or click **Open Recent Searches** to use previous search parameters.

In the **Analysis** tab:

1. Select the attribute to search for: **Relative Area**, **Relative Height**, **Area**, or **Height**.

2. Select the property to search for: Actual, Average, %CV, SD.

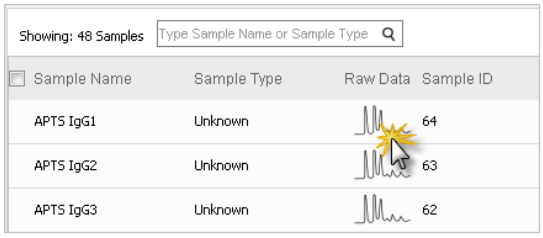
3. Specify search by value or range.

4. Specify the value (and conditions) or range to search.

5. Click **Search**.

Display sample info, raw data, or EPT data

1. In the **Processing** tab, double-click the **Raw Data** field for a sample.



   If the **Raw Data** column is not displayed, see "Create or apply table settings" on page 42.

2. Click the tab for the data of interest.

Compare processing

1. In the **Processing** tab, select a sample.
2. Assign two analysis methods to the sample by clicking the **Assign** button next to a method at the left of the screen.

3. Click **Compare Processing**.

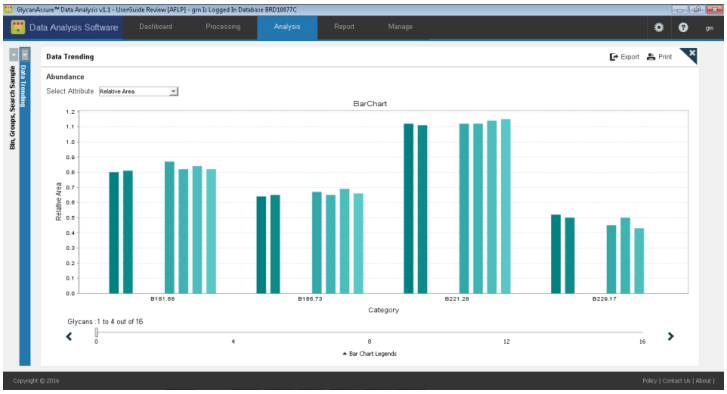
4. As needed:
   - Review the traces
   - Click **Show Differences table**
   - Select the second analysis method
   - Click **Apply to All Samples**

View data trending

Before viewing data trending, assign bins (see “Create new bins manually“ on page 23 or “Create new bins with autobinning“ on page 24).

In the **Analysis** tab:

1. Click **Data Trending** at the right of the screen to expand the pane.
   Each bar in the chart represents a bin.



2. As needed in the bar chart:
   - Click a bar to zoom on the selected bin. Click any bar to return to full view.
   - Drag the scroll bar below the bar chart to view additional bins.
   - Place the cursor on a bar for a tooltip that contains more information.

Create sample groups

You can create sample groups in the **Analysis** tab to allow easy review of subsets of samples in the project.

1. Select the samples of interest in the samples list.
2. Right-click, then select **Create Sample Group**.

Override Sizing Quality (SQ)

You can fix failed size standards in the Size Match Editor and reanalyze the samples that failed sizing.

1. In the **Processing** tab, select a sample with a failed SQ.
2. Click **SQ Editor**.
3. Adjust size standard peaks as needed, then click **Override SQ**.
   After overriding the SQ values:
   - The SQ flags are changed to .
   - Analysis status changes from to .
   - A appears in the SQI (Size Quality Invalid) Samples table column (if displayed).
4. In the **Processing** tab, click **Analyze**.

Analysis flags

Table 3 Analysis flags

Flag	Description	Status	Analysis method setting for threshold or range Analysis> Peak Calling & Quality
SQ	Sizing Quality	SQ value is in the Pass range SQ value is between the Pass range and the Low Quality range SQ value is In the Fail range	SQ Range
BD	Broad Peak	No peaks exceed the Max Peak Width threshold. One or more peaks exceed the Max Peak Width threshold.	Max Peak Width
EB	Empty Bin	Peaks are assigned to all the bins specified in the analysis method One or more bins does not have a peak assigned to it	N/A
BIN	Out of Bin	All detected peaks are assigned to bins and are identified One or more detected peaks are unassigned and are un-identified	N/A
OS	Off Scale	No fluorescence signals within the analysis range exceed the signal threshold of the instrument One or more fluorescence signals within the analysis range exceed the signal threshold of the instrument	N/A
SQI	Sizing Quality Invalid	Override SQ applied	N/A
SPU	Spectral Pull Up	No pull-up peaks are detected in the sample signal One or more pull-up peaks are detected in the sample signal	Pull up ratio
OMIT	Sample manually omitted from analysis	Sample is omitted from results calculation by the user Displays sample file name of omitted sample	N/A

Plot toolbar

List, button, or icon	Description
Plot units list	Select **Datapoints**, **RMU**, or **Migration Time**.
Select/Edit list	Select **Peak Selection**, **Edit Delimiters** (peak start/end), or **Edit Baseline**.
	Bin width selector. Active only when **RMU** and **Create/Edit Bin** are selected.
Peak display list	Select **All Peaks**, **Assigned Peaks** (peaks that are assigned to bins), and **Unassigned Peaks** (peaks that are not assigned to bins).
	Select a peak attribute: • **Offscale** pink marker • **Edited Peak** ★ marker • **Delimiter** peak start/end or **Baseline** Select a peak label: • **None** • **Bin Name** • **Area** or **Relative Area** (Actual area of peak)/(sum of area of all identified peaks in respective sample) × 100 • **Height** or **Relative Height** (actual peak height/sum of height for all identified peaks in trace) × 100 • **RMU value** • **Peak Tick** (displays # above all peaks that exceed the Peak Amplitude Threshold in the analysis method)
Autoscale	Autoscale to the tallest peak in the zoomed area.
	Unzoom and autoscale to the the tallest peak in the trace.
	Unzoom and fit plot to window.
	Zoom in and out. You can also click-drag on the plot to zoom.
	Save plot settings.

List, button, or icon	Description
	Save the plot as PNG or PDF.
	Print plots.
	Overlay or separate electropherograms.
	Dye colors to display in the plot.
	Move the zoomed area to the previous or next bin.

Examine the size standard plot and curve

To investigate SQ flags:

1. In the **Processing** tab, select the samples of interest.
2. Click **SQ Editor** to view the size standard and peak assignments.
3. Ensure that all size standard peaks are present and labeled correctly. If all peaks are not present, make sure the analysis range in the analysis method is set correctly.
4. If the peak assignments are correct, you can override the size quality value.
5. Select the Size Calling Curve tab to view the size standard curve for the selected sample.

   Red data points represent the fragments from the size standard against a black best-fit curve.
6. Click **Apply**, then **OK**.

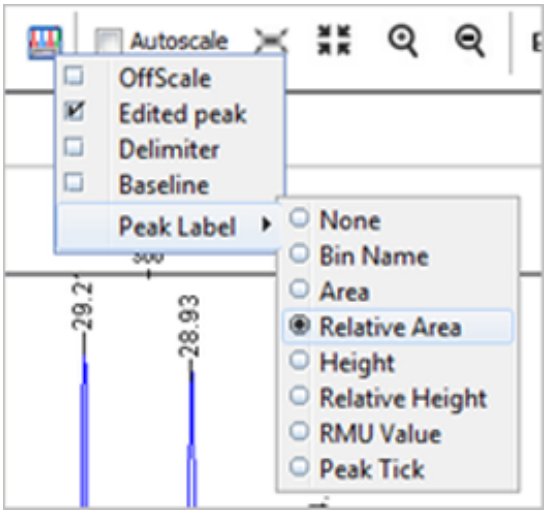
Specify peak labels

Set the peak label to display

Before selecting a peak label, assign bins (see “Create and manage bins“ on page 23).

In the **Analysis** tab:

1. Click , then select **Peak Label**.



2. Select the peak label to display.
   - For all labels except **Bin Name**,**RMU Value**, and **Peak Tick**, labels are displayed for individual peaks.
   - For **Bin Name**, labels are displayed only for peaks that are within bins.
   - For **RMU Value**, labels are displayed only for peaks that are detected with all settings specified in the analysis method.
   - For **Peak Tick**, "#" is displayed for peaks that are above the **Peak Amplitude Threshold** specified in the analysis method or specified during autobinning, regardless of the RMU range.

   Note: A (P2) peak label is automatically assigned to peaks that are detected with a Peak Amplitude Threshold lower than the threshold in the analysis method (assigned during autobinning). For more information, see “Create new bins with autobinning“ on page 24).

Remove P2 peak labels

A (P2) peak label is automatically assigned to peaks that are detected with a Peak Amplitude Threshold lower than the threshold in the analysis method (assigned during autobinning). For more information, see “Create new bins with autobinning“ on page 24).

Click **Assign Empty Bins** in the left pane, then click **Remove Second Pass**.

Print, export, or save (.pdf, .png) results

- To print, export, or save results as .pdf – Click  or  in the table toolbar.
- To print or save plots as .pdf or .png– Click  or  in the plot toolbar.
- To print or save data trending results as .pdf or .png– Click  or  in the **Data Trending** screen.

5 Manage resources

Manage projects

Edit project name or description

1. In the **Processing** tab, click ✎.
2. Edit the name or description, then click **Save**.

Define sample path

When you add samples from sample files to a project:

- You specify the location of the sample files on the hard drive or a network drive.
- Sample files remain in their original location on the drive, and are not stored in the database.
- The software reads the information it needs from the sample files. No information is written back to the original sample files.

If the sample files are later moved from their original location, you must define a new sample file path in the project if you want to view raw data, view EPT data, or reanalyze samples.

1. In the Processing tab, select the samples for which you want to define a path.
   The SFNF (Sample File Not Found) column is ■ if the software cannot find a sample file.
2. Click **Define sample path**.
3. Navigate to the original folder from which the sample was added to the project.
   The individual sample files are not listed if you open the folder containing the sample files. Select the folder, then click **Define**.
   The SFNF (Sample File Not Found) column is ■ if the software successfully locates the sample file.

Set a project as a favorite

In the **Dashboard** or **Processing** tab, click ☆.

You can search for favorites as described in "Search for a project" on page 12.

Save a copy of a project

In the **Manage** tab:

1. Click **Projects** in the left pane.
2. Select a project, then click **Save As**.

Export a project

In the **Manage** tab:

1. Click **Projects** in the left pane.
2. Select a project, then click **Export**.
3. Specify a location and a name for the exported project.
   The name of the exported project will be assigned to the project if you import it back in to the software.
4. *(Optional)* Select:
   - **Autoname** to select a name and location for the export. Otherwise the project is exported to the default location specified in preferences.
   - **Export reference data** to include the analysis method and size standard in the exported project.
   - **Overwrite existing files** to specify that the project can overwrite and existing project in the specified location.
   - **Export fsa files** to include the sample files in the exported project.
5. Click **Save**.
   A .zip file is saved to the specified location.

Import a project

In the **Manage** tab:

1. Click **Projects** in the left pane.
2. Click **Import**.
3. *(Optional)* Select **Reference data overwrite** to import the analysis method and size standard definition from the project and overwrite existing items.
4. Select the project .zip to import.
   The project is imported and assigned the name of the original project with a suffix of "Imported". If specified in the export, the following are also imported:
   - Analysis methods
   - Sample .fsa files (saved to <InstalledLocation\AppliedBiosystems\GlycanAssureDataAnalysis\Imported Projects\>)

Delete a project

In the **Manage** tab:

1. Click **Projects** in the left pane.
2. Select a project, then click **Delete**.

   The project is deleted. The analysis methods associated with the project are retained. The sample .fsa files are *not deleted* from the computer.

Manage analysis methods

Create an analysis method

1. In the **Processing** tab in the Analysis method list, click .
2. Enter parameters (see "Analysis method parameters" on page 16).
3. As needed, select **Set as default** or **Add to Favorite**.
4. Click **Save**.

Open an analysis method

In the Processing tab, click an analysis method name in the analysis method list.

Save a copy of an analysis method

In the **Manage** tab:

1. Click **Analysis Methods** in the left pane.
2. Select an analysis method, then click **Save As**.

View or edit an analysis method

In the **Manage** tab:

1. Click **Analysis Methods** in the left pane.
2. Double-click an analysis method.
3. View or edit the analysis method. Click **Save** if you edit the analysis method. The analysis status of any samples that use the edited analysis method is set to .

Export an analysis method

In the **Manage** tab:

1. Click **Analysis Methods** in the left pane.
2. Select an analysis method, then click **Export**.
3. Specify a location and a name for the exported analysis method.

   The name of the exported analysis method will be assigned to the analysis method if you import it back in to the software.
4. Click **Export**.

   A .xml file is saved to the specified location.

Import an analysis method

In the **Manage** tab:

1. Click **Analysis Method** in the left pane.
2. Click **Import**.
3. Select the analysis method .xml to import, then click **OK**.

Delete an analysis method

In the **Manage** tab:

1. Click **Analysis Methods** in the left pane.
2. Select an analysis method, then click **Delete**.
   The analysis method is deleted.
   The analysis status of any samples in an open project that use the deleted analysis method is set to .

Manage table settings

Create or apply table settings

1. Click .
2. Click **Create New**, enter a name, then click **OK**.
3. Select the columns to display.
4. Click **OK**.

Column names

Sample ID is an internal identifier applied by the software.

UD1 through UD 3 are user-defined columns that can contain information from the GlycanAssure™ Data Acquisition Software.

See “Processing flags“ on page 14 and “Analysis flags“ on page 34 for a description of flag columns.

Import, export, save a copy, or delete table settings

In the **Manage** tab:

1. Click **Table Settings** in the left pane.
2. Select a row in the table.
3. Click **Import**, **Export**, **Save As**, or **Delete** as needed.

Manage size standards

Create a size standard definition

1. In the **Processing** tab, open an analysis method, then click **Alignment**.
2. Click ➕.
3. Enter a Name and optional Description.
4. Select a Size Standard Dye.
5. Enter Peak sizes (RMU).
6. Click **Save**.

Import, export, save a copy, or delete a size standard

In the **Manage** tab:

1. Click **Size Standards** in the left pane.
2. Select a row in the table.
3. Click **Import**, **Export**, **Save As**, or **Delete** as needed.
   The analysis status of any samples in an open project that use the deleted size standard is set to .

Manage panels

Create a panel

In the **Analysis** tab:

1. Assign bins (see “Create and manage bins“ on page 23).
2. Click **Add to Library**.

Import, export, save a copy, or delete a panel

In the **Manage** tab:

1. Click **Panel** in the left pane.
2. Select a row in the table.
3. Click **Import**, **Export**, **Save As**, or **Delete** as needed.

6 Use security, audit, and e-signature (SAE) functions

The SAE functions are available if your system includes the GlycanAssure™ Security, Audit, and E-signature (SAE) Administrator Console (SAE Admin Console).

Administrators overview of SAE functions

The SAE Admin Console provides the following SAE functions for administrators.

Note: This section provides a brief overview of the functions that the SAE IT Administrator or SAE System Administrator can perform in the SAE Admin Console. For more information, see the *GlycanAssure™ Security, Audit, and E-signature (SAE) Administrator Console v1.0 Help* (Pub. No. MAN0016774) and *GlycanAssure™ Security, Audit, and E-signature (SAE) Administrator Console v1.0 User Guide* (Pub. No. MAN0016773).

Function	Description	Administrator permissions in the SAE Admin Console
Security	Controls user access to the GlycanAssure™ Data Acquisition Software and GlycanAssure™ Data Analysis Software. A default System Administrator user account is provided at installation.	• Create additional user accounts for the Data Acquisition Software and Data Analysis Software. • Set security policies: Password expiration, allowed login attempts, session lockout (the software remains idle for a specified period). • Set password policies: Password length, required characters, and use of previous passwords

Function	Description	Administrator permissions in the SAE Admin Console
Audit	Automatically tracks the following: • **Data Acquisition Software**—Tracks changes made to objects and actions performed by users. • **Data Analysis Software**—Tracks changes made to objects and actions performed by users. • **SAE Admin Console**—Tracks changes made to the SAE settings.	View and generate audit logs. The logs contain detailed information about the audited events.
Electronic signature (e-signature)	Controls user requirements to e-sign (provide a user name and password) the following objects: • **Data Acquisition Software**—Instrument methods • **Data Analysis Software**—Projects and project reports	• Grant e-signature authority to user accounts. • Enable e-signatures for the following objects: Instrument methods, projects, and/or project reports, and configure the number of e-signatures required. • Create e-signature reasons. • View, generate, and export e-signature logs. The logs contain detailed information about the e-signature events.

Users overview of SAE functions

The SAE Admin Console controls the following SAE functions for users in the GlycanAssure™ Data Analysis Software.

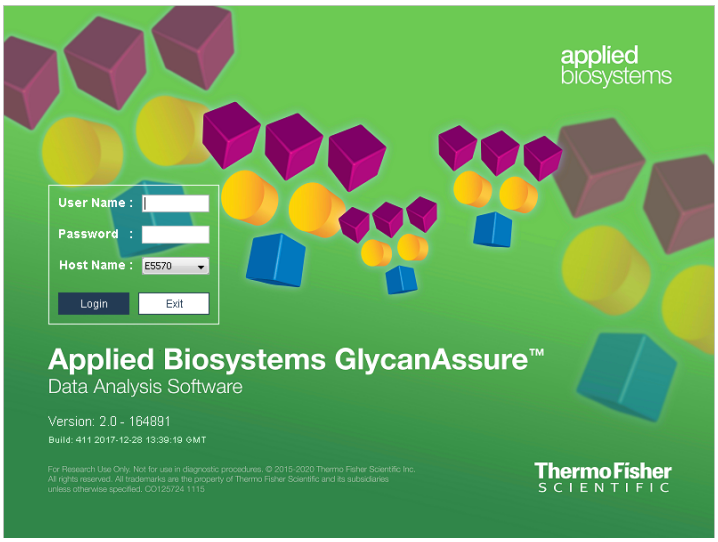
Function	Description	User permissions in the Data Analysis Software
Security	Controls user access to the GlycanAssure™ Data Analysis Software.	• Log in to and exit the software. • View the user profile. • Lock a session. • Change expired passwords. See "Security" on page 46.

Function	Description	User permissions in the Data Analysis Software
Audit	Automatically tracks changes made to objects and actions performed by users.	View and export audit logs. The logs contain detailed information about the audited events. See "Audit" on page 49.
Electronic signature (e-signature)	Controls user requirements to e-sign (provide a user name and password) projects and project reports. Once a project or project report has been completely e-signed, it cannot be modified or deleted. However, you can create additional project reports for a project, then modify the new report as needed.	• E-sign projects and project reports. • View and export e-signature logs. The logs contain detailed information about the e-signature events. See "Electronic signature" on page 51.

Security

Log in

Enter your user name and password to access the software.

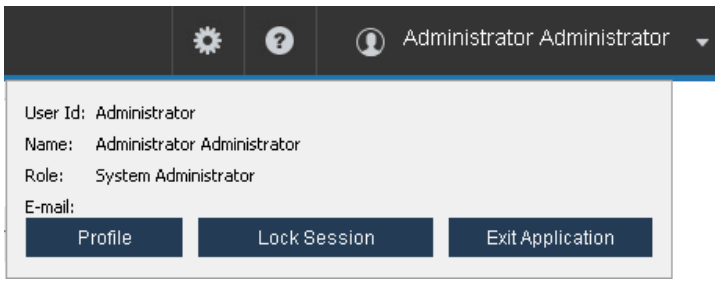


Your access to functions in the software is based on the permissions associated with your user account. Functions for which you do not have permissions are dimmed.

If your system is configured for password expiration, you will be periodically prompted to change your password. If your system is configured to monitor failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password more than a specified number of times.

Exit

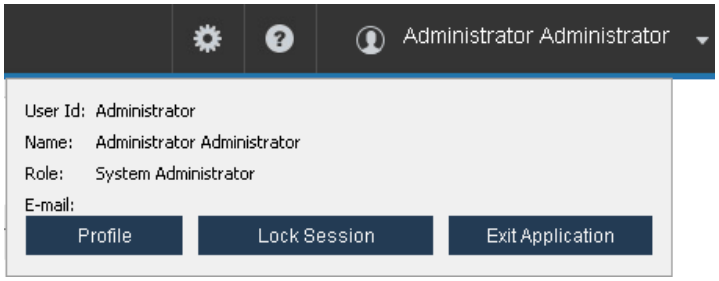
1. Click the name of the logged in user in the far right of the menu bar.



2. Click **Exit Application**.

View and change the user profile

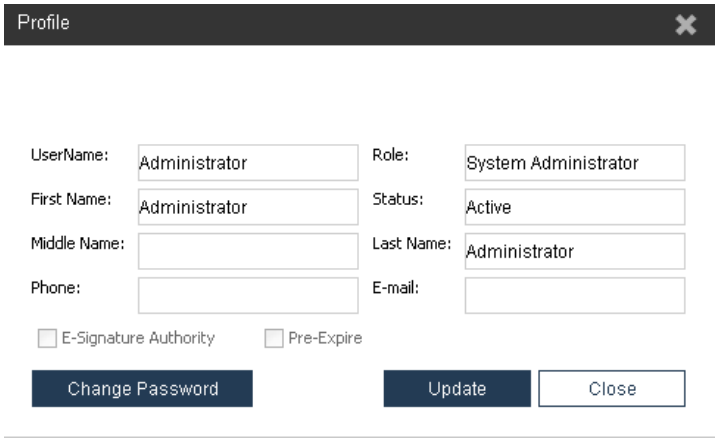
1. Click the name of the logged in user in the far right of the menu bar.



2. Click **Profile**.

3. Change the profile as needed, click **Update**, then close the **Profile** dialog box.

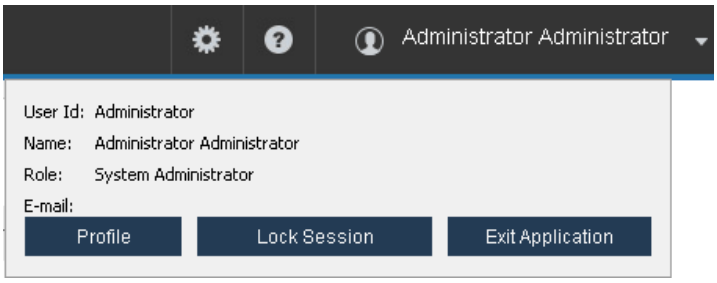
   Note: You cannot change the **User Name**, **Role**, **Status**, **E-Signature Authority**, or **Pre-Expire**.



Lock a session

If you need to leave the software running while you are away, you can lock a session. The software continues to run the session, but other users will not be able to access the session.

1. Click the name of the logged in user in the far right of the menu bar.

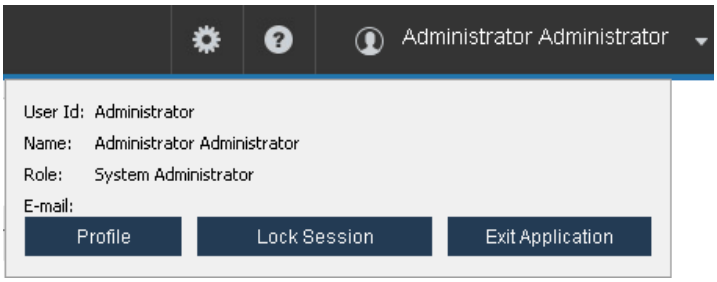


2. Click **Lock Session**, then click **Yes** to confirm.

Change your password when it expires

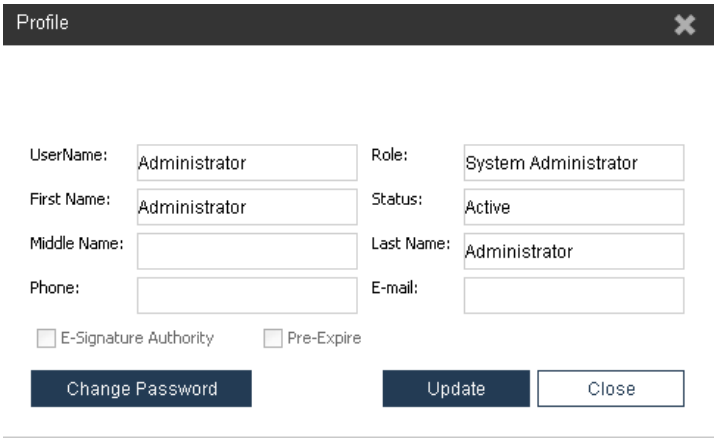
When your password is about to expire, a message is displayed when you log in.

1. Click the name of the logged in user in the far right of the menu bar.



2. Click **Profile**.

3. Change your password:
   a. Click **Change Password**.



   b. Enter the old password.

c. Enter a new password, confirm the new password, then click **OK**.

Audit

View, generate, and print object audit logs

1. In the **SAE** tab, click **Audit Log**, then click the **Objects** tab.
2. Click **Refresh** to synchronize the SAE Admin Console with the Data Analysis Software.
3. *(Optional)* Click **Filters**, select or enter the filter criteria as needed, then click **Apply Filter** to display the filtered list.

To filter by the...	Do this...
Date range	In the **From** and **To** fields, click the **(calendar)**, then select a date
Record name	In the **Record** field, enter a record name. Partial entry is sufficient (for example, if you enter **IgG**, all record names that include **IgG** are displayed).
User name	In the **Username** field, enter a user name. Partial entry is sufficient (for example, if you enter **Smith**, all user names that contain **Smith** are displayed).
Object type	Select an object **Type**: • **Analysis Method** • **Size Standard** • **Project** • **Panel**
Actions performed	Select an **Action**: **Note:** The list of actions varies, depending on the object type that you selected. • **Create** • **Update** • **Delete**

Note: To remove the filters, click **Reset Filter**.

4. Generate or print the log:

If you want to...	Then...
Generate summary information	Click **Summary Report** to generate and open a .pdf file.
Generate detailed information	Select the objects of interest in the list, then click **Detailed Report** to generate and open a .pdf file.
Print the log	Click **Print**.

View, generate, and print event audit logs

1. In the **SAE** tab, click **Audit Log**, then click the **Events** tab.
2. Click **Refresh** to synchronize the SAE Admin Console with the Data Analysis Software.

3. *(Optional)* Click **Filters**, select or enter the filter criteria as needed, then click **Apply Filter** to display the filtered list.

To filter by the...	Do this...
Date range	In the **From** and **To** fields, click the **(calendar)**, then select a date
Record name	In the **Record** field, enter a record name. Partial entry is sufficient (for example, if you enter **admin**, all record names that include **admin** are displayed).
User name	In the **Username** field, enter a user name. Partial entry is sufficient (for example, if you enter **Smith**, all user names that contain **Smith** are displayed).
Event type	Select an **Event Type**: • **System** • **Panel** • **Size Standard** • **User** • **Project** • **Analysis Method**
Actions performed	Select an **Action**: **Note:** The list of actions varies, depending on the event type that you selected. • **Login** • **Logout** • **Login Failed** • **Password Changed** • **Manual Binning** • **E-signature** • **Apply Alignment** • **Password Expired** • **Create** • **Apply Auto Binning** • **Analyze Samples** • **User Authentication** • **License Set** • **Update** • **Session Timeout**

Note: To remove the filters, click **Reset Filter**.

4. Generate or print the log:

If you want to...	Then...
Generate summary information	Click **Summary Report** to generate and open a .pdf file.
Print the log	Click **Print**.

Electronic signature

Guidelines for multiple e-signatures

If an object requires two or more e-signatures:

- The signers are not required to sign at the same time.
- When the first signer signs, the icon next to the **e-sign** button changes to , and the **Signed Status** in the **Manage** tab is set to **Partially Signed**.
- When all required signers sign, the icon next to the **e-sign** button changes to , and the **Signed Status** in the **Manage** tab is set to **Signed**.

E-sign a project

If your system is configured for electronic signature, you can optionally e-sign projects.

1. In the **Analysis** tab, click **e-sign**.
2. Select a reason for the e-signature, enter your user name and password, then click **Apply**.

The icon next to the **e-sign** button changes to , and the **Signed Status** in the **Manage** tab is set to **Signed**.

Note: If two or more e-signatures are required, see "Guidelines for multiple e-signatures" on page 51.

E-sign a project report

If your system is configured for electronic signature, you can optionally e-sign project reports for projects that are completely e-signed.

1. In the **Report** tab, select the **Signed Report** option.
2. Select the report components, then click **Create**.
3. Enter your user name and password, then click **Apply**.

When the project report is generated, a detailed list of the e-signatures is included at the bottom of the report.

View, generate, export, and print e-signature logs

E-signature logs contain e-signature records from the GlycanAssure™ Data Analysis Software.

The log information is automatically recorded by the software and cannot be modified.

1. In the **SAE** tab, click **E-signature Log**.
2. Click **Refresh** to synchronize the SAE Admin Console with the Data Analysis Software.

3. *(Optional)* Click **Filters**, select or enter the filter criteria as needed, then click **Apply Filter** to display the filtered list.

To filter by the...	Do this...
Date range	In the **From** and **To** fields, click the **(calendar)**, then select a date
Reason for the e-signature	In the **Reason** field, enter a reason. Partial entry is sufficient (for example, if you enter **review**, all reasons that include **review** are displayed).
User name	In the **Username** field, enter a user name. Partial entry is sufficient (for example, if you enter **Smith**, all user names that contain **Smith** are displayed).
Object type	Select an **Object Type**: • **Project**
Object name	In the **Object Name** field, enter an object name. Partial entry is sufficient (for example, if you enter **Demo**, all object names that contain **Demo** are displayed).

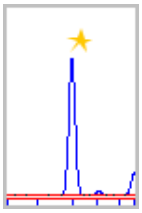
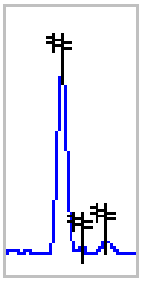
Note: To remove the filters, click **Reset Filter**.

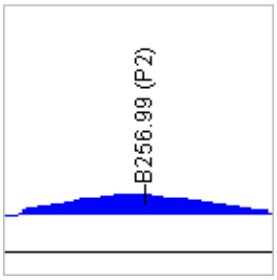
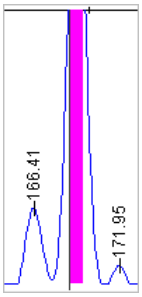
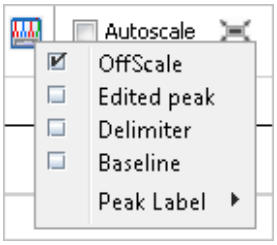
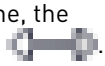
4. Generate, export, or print the log:

Option	Description
Generate summary information	Click **Summary Report** to generate and open a .pdf file.
Export the log	Click **Export** to export a .csv, .pdf, or .xls file to a location of your choosing.
Print the log	Click **Print**.

7 Troubleshooting

Troubleshooting

Observation	Possible cause	Action
"Some samples are not normalized. A normalization factor will not be applied to un-normalized samples" message.	One or more .fsa files was acquired with a size standard that does not specify normalization, but the analysis method has Enable Size Standard Normalization enabled in the analysis method (Processing> Alignment).	Only samples acquired with a size standard that specifies normalization will be normalized during processing.
"Assigning bin to peak failed" message.	Peak is already assigned to bin	No action.
	Peak is not a detected peak (no start and end tick marks displayed).	Adjust peak detection if needed ("Adjust delimiters (peak start/end) and baseline" on page 29).
In the **Processing** tab, the **Analyze** button is dimmed.	Analysis method is not assigned to at least one sample.	Assign analysis method.
Peak is flagged with a star. 	Peak delimiters (start, end) or baseline were manually adjusted.	No action.
Peak is flagged with a # (number sign or hash tag). 	The Peak Tick label is selected (> Peak Labels>Peak Tick) and displays # above all peaks that are above the peak amplitude threshold in the analysis method or the Peak Amplitude Threshold used for second pass autobinning.	No action.

Observation	Possible cause	Action
Peak is flagged with **(P2)**. 	The peak was detected during second pass autobinning.	No action.
Peak is flagged with pink marker. 	The peak is offscale, and the Offscale peak marker is selected in the plot. 	No action.
When adjusting the baseline, the cursor does not change to  .	High magnification is required to adjust the baseline.	Zoom in on the y axis. You may need to zoom to <500 RFU.

Documentation and support

Related documents

The following related documents are available:

Document	Publication Number
GlycanAssure™ Data Acquisition Software v2.0 Help	MAN0014719
3500/3500xL Genetic Analyzer with GlycanAssure™ Data Acquisition Software v2.0 User Guide	100036372
GlycanAssure™ Data Analysis Software v2.0 Help	MAN0014720
GlycanAssure™ Data Analysis Software v2.0 User Guide	100036373
GlycanAssure™ Security, Audit, and E-signature (SAE) Administrator Console v1.0 Help	MAN0016774
GlycanAssure™ Security, Audit, and E-signature (SAE) Administrator Console v1.0 User Guide	MAN0016773
GlycanAssure™ System Quick Reference	100038224

Note: For additional documentation, see "Customer and technical support" on page 55.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

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

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Index

O

P

R

S

T

U

W

Z