

LEGENDplex™

Data Analysis Software Suite Manual

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Program Overview:

Introduction

The LEGENDplex™ Data Analysis Software Suite is designed to analyze LEGENDplex™ flow cytometry data files, and was developed in collaboration with Qognit.

The software suite consists of both an online cloud-based program as well as downloadable desktop applications for both PC and Mac computers.

Key software features include:

- A simple and intuitive user interface and workflow.
- Pre-loaded Beads IDs for all pre-defined and mix and match panels.
- A sophisticated gating and curve-fitting algorithm that analyzes each flow cytometry file individually to ensure result accuracy.
- Easy generation of summary reports for downstream analysis.
- Simple import/export of experiment workspaces between the cloud and desktop programs

Cloud-Version Specific Features:

- Ability to return to the analysis workspace at any time after uploading assay FCS files.
- Easily share any assay workspace with collaborators or BioLegend technical service.
- 2 GB account space on the server, allowing dozens of assays worth of data to be stored simultaneously.

Account Registration

To request access to the LEGENDplex™ Data Analysis Software Suite, send an email to BioLegend's technical service team. Please use the following information when drafting your email:

- Email recipient: tech@biolegend.com
- Subject line LEGENDplex™ Data Analysis Software Suite Account Setup
- Email body: Please mention that you would like to have an account setup for the LEGENDplex™ Data Analysis Software Suite

Note: Be sure to include the email address that you would like to use for account access. This email will be used for both the Cloud and the Desktop Application programs.

A confirmation email, informing users that their account has been activated, will be sent to the address listed in the email body within 1 business day.

Please follow the instructions included in this email to set your account password.

Note: In order to ensure that your Desktop version can display panels from the Cloud, your password must be identical for both the Cloud and Desktop versions.

All programs within the LEGENDplex™ Data Analysis Software Suite are compatible with the following flow cytometer file types:

- FCS 2.0
- FCS 3.0
- FCS 3.1
- LMD files

Data Security

Registered account users retain full control of any data they upload to the cloud servers. Account owners have sole permission rights to delete or add data sets to the program. At no time will any other user be able to access your data unless expressly granted permission to do so by you via the share functionality in the program. This permission can also be revoked at any time.

Qognit hosts the LEGENDplex™ software on virtual servers provisioned on world class, leading cloud providers like Amazon EC2, Google Cloud, and Digital Ocean. Qognit will only use cloud providers with data centers that are SOC 2 accredited, and who implement physical security safeguards which at minimum have:

- 24/7 physical security guard services & monitoring
- Physical entry restrictions to their facilities
- Biometric readers with two-factor authentication
- CCTV coverage of the facility

All incoming and outgoing data transfers through the LEGENDplex™ software are encrypted during transit using modern, secure TLS (HTTPS) cryptographic protocols.

Access Logging: Server and Application level access (both by Qognit and Customers) is logged to a central logging system. The logs are monitored and flagged for unusual and potentially malicious activities, and all flagged activity is investigated by Qognit or our designated security vendor.

Penetration testing and vulnerability management: Qognit will regularly perform internal and external penetration tests of the LEGENDplex™ software, and will appropriately remedy any identified issues based on their severity. Qognit actively monitors emails sent to security@qognit.com and investigates all reported potential vulnerabilities and bugs.

Breach Notification: Since no method for transmitting and storing electronic data is perfectly secure, Qognit cannot provide a guarantee of absolute security. If Qognit learns of a security breach, they will notify the affected customers so that they can take the appropriate steps for handling such a breach. Our notification procedures are, at minimum, compliant with applicable regulations and industry standard practices.

Program Access

After completing the account registration process, users should complete the following steps to access the software:

Cloud-based Program

- Navigate to legendplex.qognit.com using your preferred web browser.
- (Optional) Access the program via the 'Cloud-based Program' section found under the software tab on the LEGENDplex™ webpage (biolegend.com/en-us/legendplex).
- Enter your email address and password into the log in window (Figure 1).
- Click the purple 'Login' button to access the software.

Desktop Applications

- Log in to the cloud-based program.
- Click the purple bust icon located next to your email address in the upper right quadrant of the screen.
- Select either 'Download Windows Desktop Application' or 'Download MacOS Desktop Application'
- Click the 'Accept Terms' checkbox for the software terms of service and click 'Submit'
- Save the zipped folder to your computer and then extract the folder to your hard drive.
- Launch the 'legendplex' app from the folder.

Note: The first time you launch the desktop application programs you will be required to grant them permission to run on your computer. The steps to accomplish this are listed below and need only be completed once.

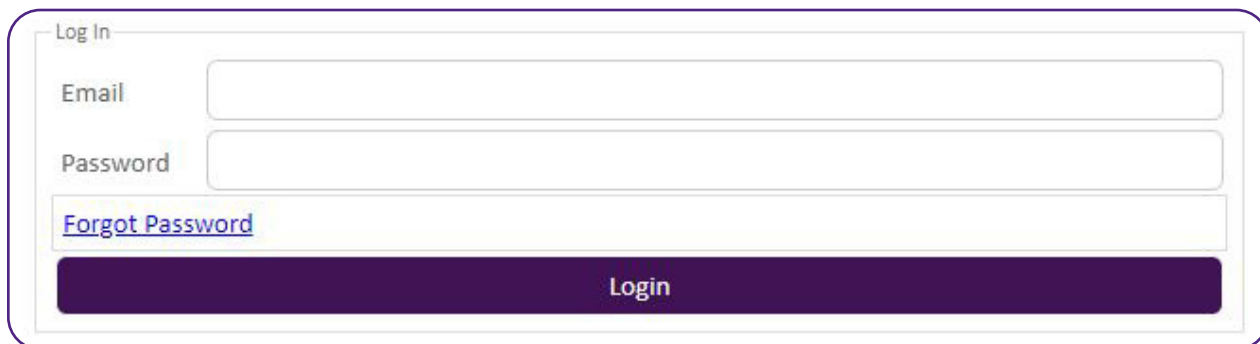
PC Computers

- Click the 'More Info' hyperlink in the unrecognized app pop-up window that appears
- Click the 'Run Anyway' button at the bottom of the pop-up window

Mac Computers

- Click the 'Open' button in the pop-up window that appears
- Confirm that a second pop-up appears informing you that the program cannot be opened
- Click the following: Apple Icon — System Preferences — Security & Privacy — General tab
- Click the 'Allow Anyway' button to the right of the "LEGENDplex was blocked from use because it is not from an identified developer."

Figure 1. Log in Window



The image shows a login window with a white background and a purple border. At the top left, it says "Log In". Below this are two input fields: "Email" and "Password". To the right of the "Password" field is a blue link that says "Forgot Password". At the bottom of the window is a large purple button with the word "Login" in white text.

Forgotten Password

If you have forgotten your password please navigate to legendplex.qognit.com, click the 'Forgot Password' link found in the log in window, and follow the provided instructions to reset your password.

Note: In order to ensure that your Desktop version can display panels from the Cloud, your password must be identical for both the Cloud and Desktop versions.

Software Landing Page (Cloud Software Only):

The landing page displays a table (Figure 2) which summarizes the details of any previously uploaded data sets. If this is the first time you are using the software, this table will be blank.

- Click on an experiment displayed in the table to access a previously analyzed data set.
- Click on the BioLegend logo at the top left of the screen to return to the landing page from anywhere in the software.

Figure 2. Software Landing Page

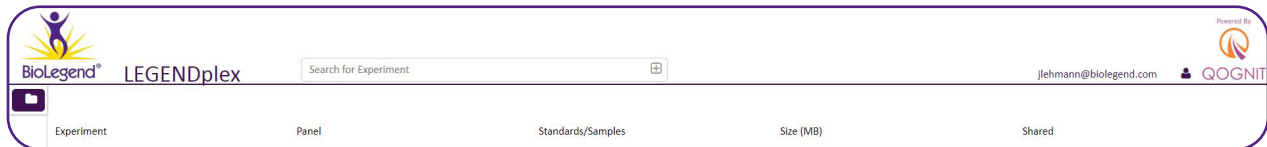


Table Column Descriptions

Experiment: Displays the user-provided name of the experiment.

Panel: Displays the LEGENDplex™ panel used in the experiment, including the plex-size of the assay.

Standards/Samples: Displays the number of FCS files identified as either standards or samples.

Size: Displays the amount of server hard drive space taken up by the assay.

Note: By default, users will have a 2 GB limit on the amount of data they can store on their account before they will be prompted to delete old assays to free up space. Please see the Data Management & Storage section of this user guide.

Shared: If you have shared the analysis workspace with another registered user, their account email address will be displayed here.

Creating a New Experiment

- Click on the purple folder icon (Figure 3) located below the BioLegend logo in the upper left of the screen.

This will expand a display menu on the left hand side of the screen that lists any uploaded assays as well as those assays which have been shared with your account by other users.

- Click the '+' icon to create a new analysis session.

Figure 3. The Folder Icon



This will open the 'New Experiment' pop-up window (Figure 4).

Figure 4. New Experiment Window



- Enter an assay name in the Experiment Name field.
- Click the 'Create+' button.

Defining Assay Parameters via Analysis Tabs:

Note: The desktop application programs opens here after entering log-in credentials rather than the landing page in figure 2.

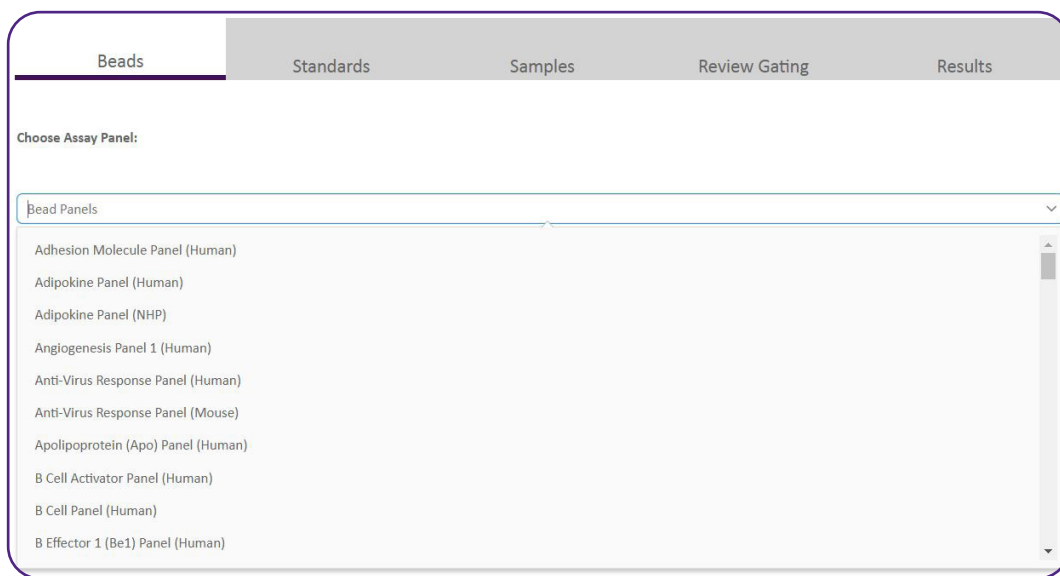
There are 5 analysis tabs: Beads, Standards, Samples, Review Gating, and Results. These tabs are sequentially used to upload and then analyze LEGENDplex™ assay FCS files. The tabs are located at the top of the page, and users can easily navigate between tabs by clicking on them, once they have been enabled by the completion of the previous step. The currently selected tab is underlined in purple. Each of the tabs, and the functionalities contained within them, are discussed below:

Beads Tab

After you create a new assay, the software will automatically navigate to the Beads tab. This tab is used to select the LEGENDplex™ panel associated with your experiment and define top standard concentrations for relevant Bead IDs.

- Choose your assay panel from the 'Bead Panels' pull down menu (Figure 5).

Figure 5. Beads Panels Pull Down Menu



The Bead IDs for the selected panel will now be displayed (Figure 6).

Notes: The Bead IDs for the full pre-defined panel will be displayed. If you are using a mix & match assay, remove any irrelevant Bead IDs by clicking the trash can icon.

For Custom LEGENDplex™ panels, please select 'Custom Panel' from the pull-down menu. Users will need to manually enter all relevant analyte names into the appropriate fields for each Bead ID in their assay. Please refer to your assay manual to locate this information. Remove any irrelevant Bead IDs by clicking the appropriate trash can icon.

- Enter the top standard concentration for each analyte in the 'Max' column.

This information can be found listed on the lot-specific certificate of analysis included with your assay.

Note: Pressing 'Tab' on your keyboard will paste the most recently entered concentration over to the next analyte, which may be useful when multiple Bead IDs share the same top standard concentration.

- Click the 'Save' button at the bottom of the Bead ID table once all the information above has been entered to proceed to the Standards Tab.

Figure 6. Beads ID Table

A Beads:					B Beads:				
Bead ID	Analyte	Max	Units		Bead ID	Analyte	Max	Units	
A4	IL-5	10000	pg/ml		B2	IFN- γ	10000	pg/ml	
A5	IL-13	10000	pg/ml		B3	TNF- α	10000	pg/ml	
A6	IL-2	10000	pg/ml		B4	IL-17A	10000	pg/ml	
A7	IL-6	10000	pg/ml		B5	IL-17F	10000	pg/ml	
A8	IL-9	10000	pg/ml		B6	IL-4	10000	pg/ml	
A10	IL-10	10000	pg/ml		B7	IL-21	10000	pg/ml	
					B9	IL-22	10000	pg/ml	

Standards Tab

The Standards Tab is used to define the number of standard curve points in your LEGENDplex™ assay, the number of replicates for each point, and the dilution-fold used to dilute assay standards.

Note: The default parameters that are displayed in these fields match those found in all LEGENDplex™ assay manuals. If you have followed these instructions, then these values do not need to be adjusted. If you elected to perform your LEGENDplex™ assay without any blank wells, please uncheck the 'Blank/s included' box.

- (Optional) Adjust the number of curve points, replicates, and dilution-fold to match those used in your LEGENDplex™ assay.
- Click the purple 'Select Standards FCS file(s)' button.
- Use the pop-up window to navigate to the location of the standard curve FCS files, select all relevant files and then click 'Open'. This will populate the file list on the Standards Tab.
- Select the fluorescence channels used to measure PE and APC signals on your cytometer from the pull down tabs below the file list.

Note: The software automatically detects scatter gating parameters. Should users wish to manually define these they should complete the following: Click the purple 'Advanced Options' button — Uncheck 'Auto-Detect Scatter Gating Parameters' — Manually select X and Y scatter.

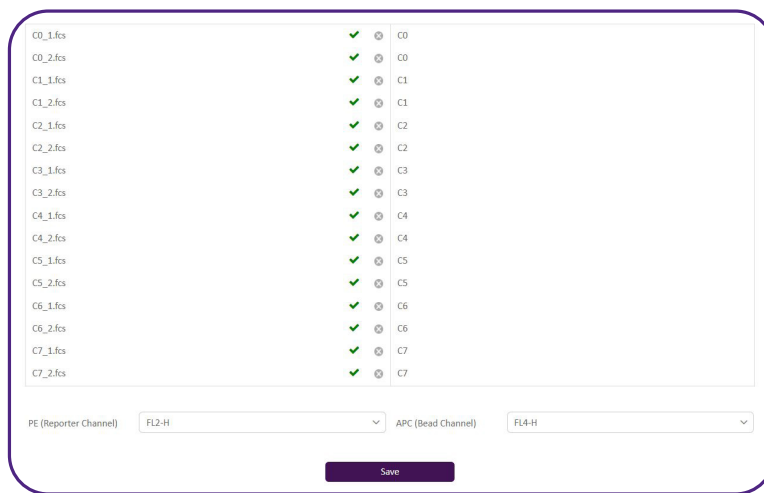
- Click the purple 'Upload' button.

Figure 7. Standards Tab

The software will now order your standard curve files from C7-C0 based on the MFI values found in the PE (Reporter Channel) identified above.

- Click and drag any FCS files that have been mislabeled to their appropriate standard curve point identifier (C0-C7) found on the right side of the table (Figure 8).
- Click the 'Save' button once all FCS files have been correctly associated with their appropriate identifier to proceed to the Samples Tab.

Figure 8. Properly Ordered Standard Curve Files



Samples Tab

The Samples Tab is used to upload the experimental sample FCS files from your LEGENDplex™ assay. This tab is also used to input any replicate and dilution information about these files.

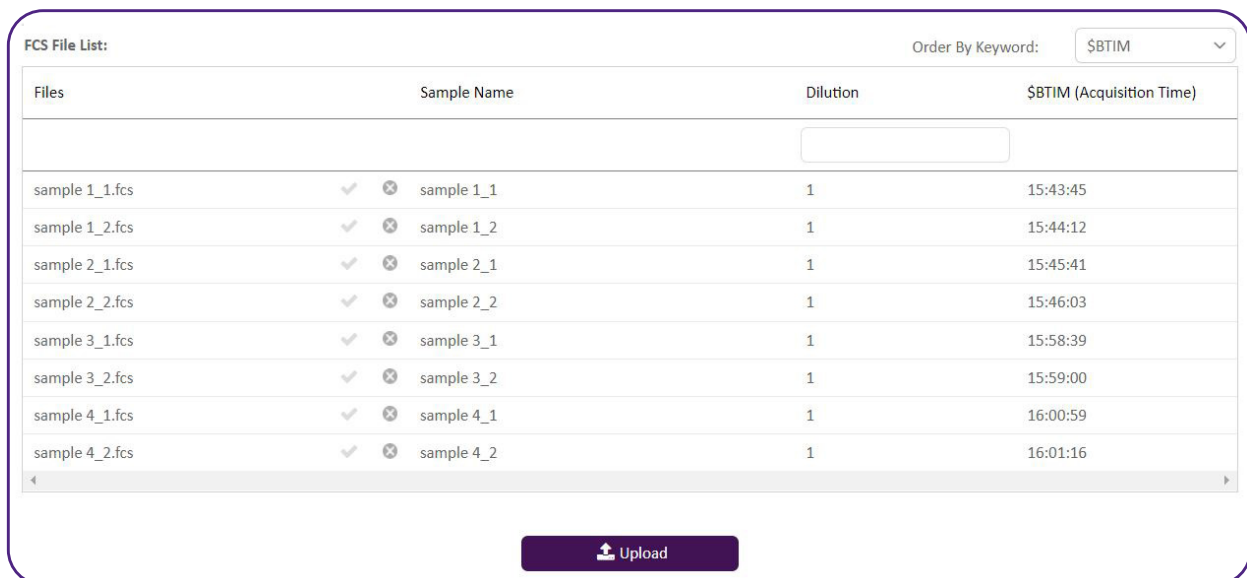
Note: If you wish to analyze only standard curve FCS files without any samples, please click the 'Run Gating' button, located to the lower right of the 'Upload' button, to proceed with your analysis using only the standards uploaded in the previous section.

- Click the purple 'Select Samples FCS file(s)' button.
- Use the pop-up window to navigate to the location of the experimental samples FCS files, select all relevant files and then click 'Open'. This will populate the FCS file list (Figure 9).

Note: The software automatically orders the selected files by their acquisition time stamp, under the assumption that replicate samples will have been acquired sequentially on the flow cytometer.

To order your sample files by another parameter, use the 'Order by Keyword' pull-down menu. Sample files can also be ordered by clicking and dragging individual files to their appropriate position on the file list.

Figure 9. FCS File List



Defining Replicates

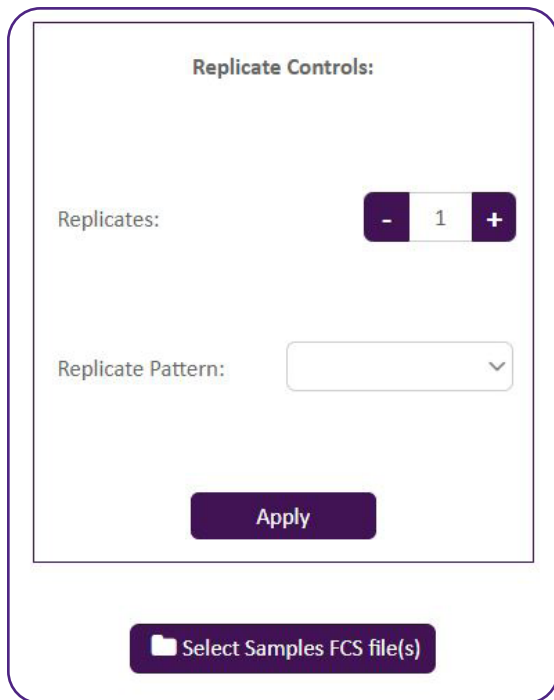
Replicates can be defined by using the Replicate Controls Box (Figure 10).

- Define the number of replicates to match your assay conditions.
- Define the replicate pattern from the pull-down menu.
- AABCC: replicate FCS files are arranged sequentially.
- ABCABC: the FCS files from the first replicate of all samples are arranged sequentially followed by the second replicate for all samples.
- Click the purple 'Apply' button in the Replicate Controls box.

This will populate the Sample Name column on the file list, using a Sample₁...Sample_n nomenclature. FCS files with the same sample name will be treated as replicates by the software.

Note: Users can manually enter their own preferred Sample Names into the appropriate fields. All FCS files containing the same Sample Name will be treated as replicates by the software.

Figure 10. Replicate Controls Box

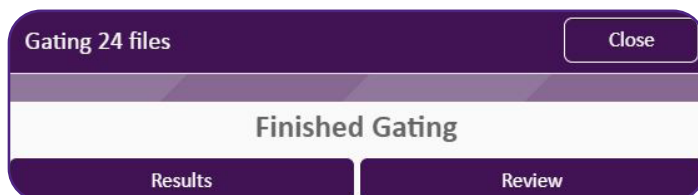


The image shows a software interface for defining replicates. It features a white box with a purple border titled "Replicate Controls:". Inside this box, there is a "Replicates:" label followed by a spinner control showing the number "1" with minus and plus buttons. Below that is a "Replicate Pattern:" label followed by a dropdown menu. At the bottom of the white box is a purple "Apply" button. Below the white box, outside its border, is a purple button with a folder icon and the text "Select Samples FCS file(s)".

- Click the purple 'Upload' button below the FCS file list.

The software will now upload and gate all standard and sample files; a progress bar will display the status of these actions.

Figure 11. Gating Progress Window



Once gating is finished users will be prompted to either view their assay results or to review gating (Figure 11).

- (Strongly recommended) Click purple 'Review' button to proceed to gating review.
- Click purple 'Results' button to view assay results without gating review.

Note: Although the software utilizes robust algorithms to gate assay files, users are strongly encouraged to review and verify these results in order to ensure quantitation accuracy.

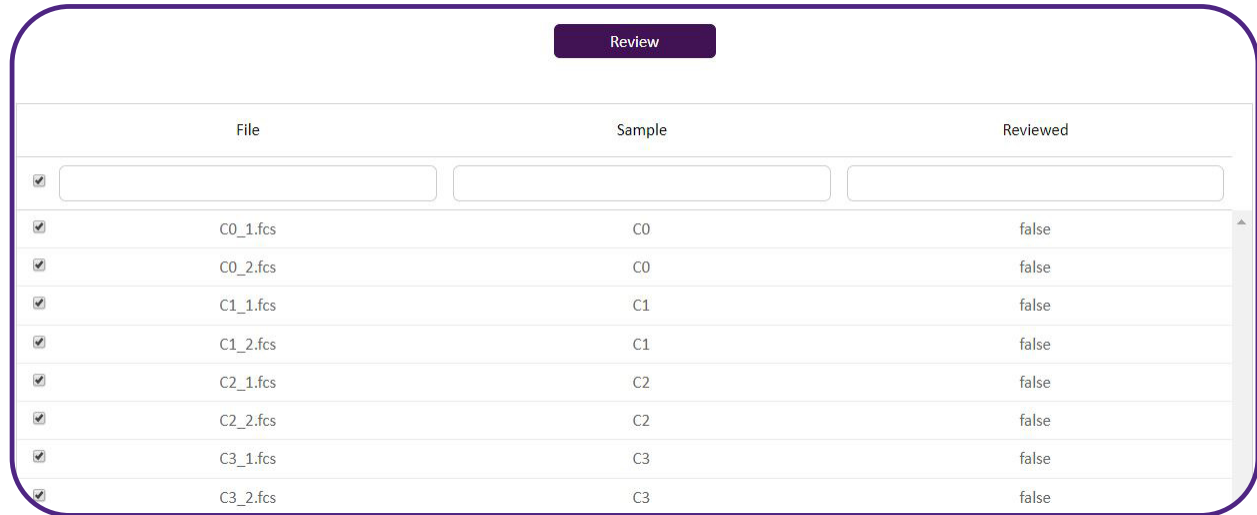
Review Gating Tab

This tab is used to review the gating results for uploaded FCS files. To review a given FCS file, the checkbox next to the file name must be selected.

Note: All uploaded files are selected by default.

- Ensure that the checkbox is checked for all files you wish to review.
- Click the 'Review' button above the file list (Figure 12).

Figure 12. Selecting Files for Gating Review



The screenshot shows a software interface with a purple header bar containing a 'Review' button. Below the button is a table with three columns: 'File', 'Sample', and 'Reviewed'. Each row in the table has a checkbox in the first column, followed by the file name, sample name, and a 'false' value in the 'Reviewed' column. The files listed are C0_1.fcs, C0_2.fcs, C1_1.fcs, C1_2.fcs, C2_1.fcs, C2_2.fcs, C3_1.fcs, and C3_2.fcs, with samples C0, C1, C2, and C3 respectively.

	File	Sample	Reviewed
<input checked="" type="checkbox"/>			
<input checked="" type="checkbox"/>	C0_1.fcs	C0	false
<input checked="" type="checkbox"/>	C0_2.fcs	C0	false
<input checked="" type="checkbox"/>	C1_1.fcs	C1	false
<input checked="" type="checkbox"/>	C1_2.fcs	C1	false
<input checked="" type="checkbox"/>	C2_1.fcs	C2	false
<input checked="" type="checkbox"/>	C2_2.fcs	C2	false
<input checked="" type="checkbox"/>	C3_1.fcs	C3	false
<input checked="" type="checkbox"/>	C3_2.fcs	C3	false

The software will then display the gating results for the first selected file from the list. The file name will also be displayed in the purple bar at the top.

3 sets of gates are displayed by the software:

1. Forward scatter vs. side scatter: Used to identify A and B bead populations.
2. A-beads classification channel mono-variate histograms: Used to identify A bead IDs.
3. B-beads classification channel mono-variate histograms: Used to identify B bead IDs.

Note: Click the toggle button above each histogram plot to switch back and forth between this view and a PE vs APC plot. This alternate view is useful for troubleshooting purposes, including investigating the presence of bead aggregates.

The number of events in each of these gates is displayed in the gating counts table below the plots.

- Click the 'Reviewed' checkbox to the upper right of the gating plots if the applied gates are correct for the FCS file. The cart icon will automatically adjust to reflect the remaining number of files to be reviewed (Figure 13a).
- Select Convert Gates to switch between 1D and 2D plots for A and B gating plots across all wells (Figures 13b, 13c)
- Click the left and right carot (< >) on either side of the gating plots to scroll through the remaining FCS assay files (Figures 13a, 13c).

Figure 13a. Gating Review Plots – 1D Plots

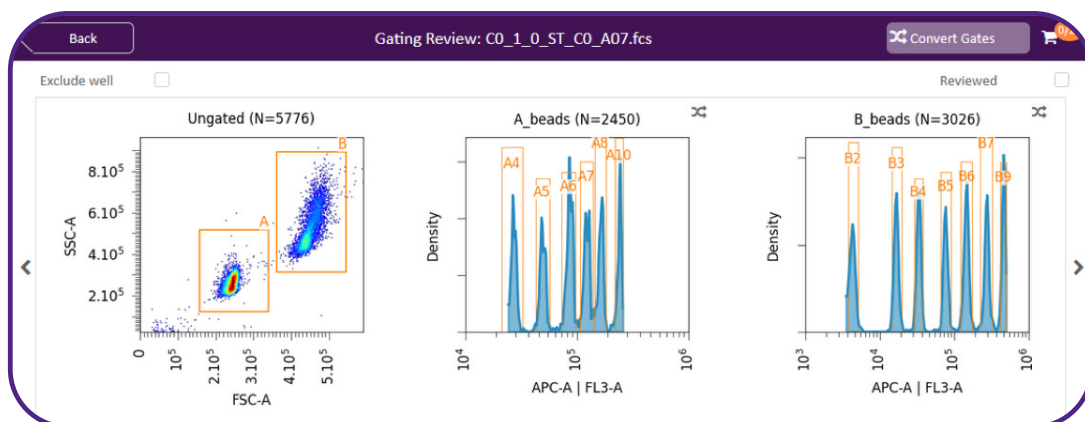


Figure 13b. Gating Review Plots – Convert Gates

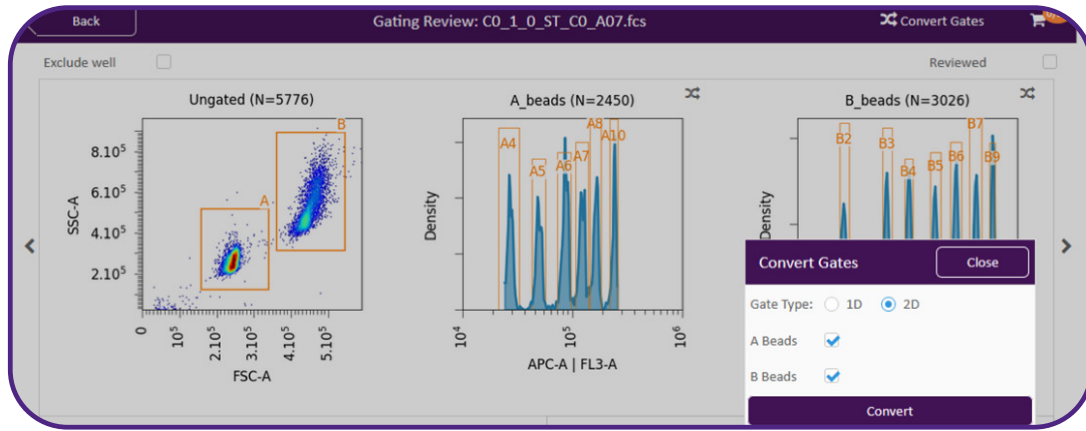
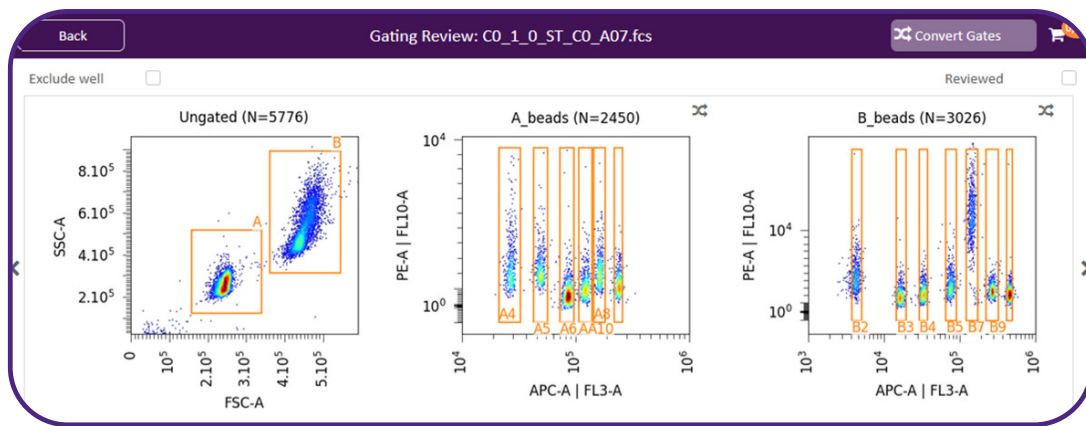


Figure 13c. Gating Review Plots – 2D Plots



Adjusting Gates

If you need to adjust any gates for an FCS file:

- Click on the set of gates you wish to adjust to open the 'Edit Gating' pop-up window (Figure 14).
- Make any adjustments necessary to draw discrete gates for each bead population.
- Click the 'Save' button to save any changes to gates; otherwise click 'Close'.

Figure 14. Edit Gating Window

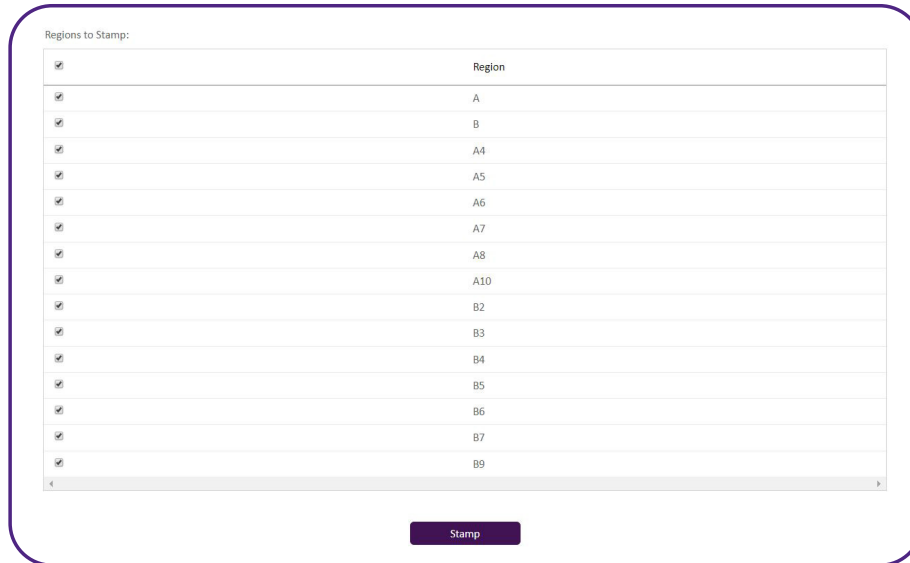


Gate Stamping

Should you wish to apply the newly adjusted gating strategy to the rest of the files from your LEGENDplex™ assay, please perform the following:

- Click the 'Apply Gating' button found below the gating counts table.
- Select which gates you would like to stamp to your other files from the list that appears (Figure 15).
- Click the 'Stamp' button when finished and the software will apply this new gate to all assay files.

Figure 15. Stamping Gates



- Click the Results tab at the top of the page once all files have been reviewed to proceed to your assay quantitation results.

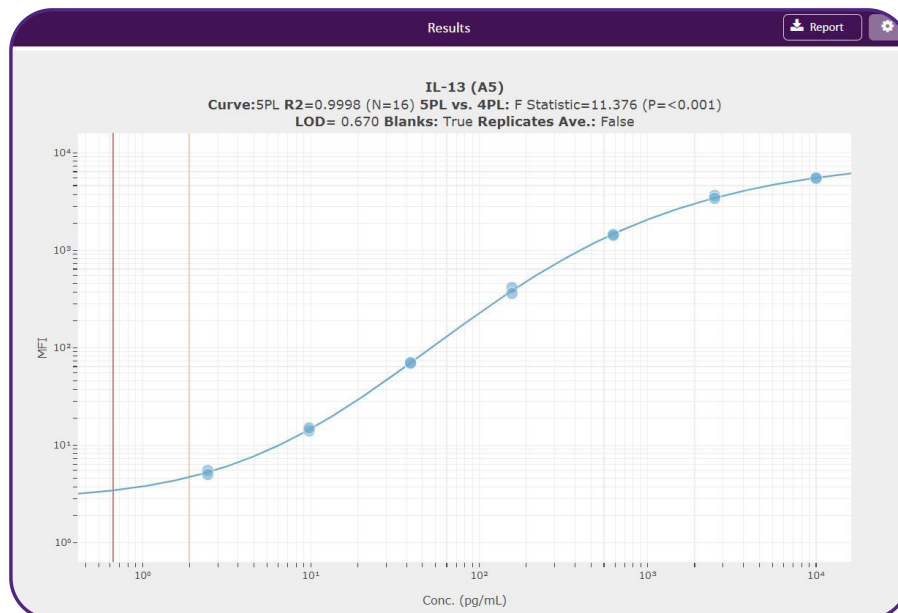
Results Tab

The analysis results from your LEGENDplex™ assay are displayed on this tab. The results are presented with assay standard curve plots above the tabular quantitation results.

Standard Curve Plots

For each analyte in your LEGENDplex™ assay, the software fits a 5-PL curve to the uploaded FCS standard curve files using logistic regression curve fitting algorithms. These curves are then used to calculate analyte concentrations in the uploaded experimental samples.

Figure 16. Standard Curve Graph



Toggle between analyte standard curve plots by clicking on the cell containing the name of the desired analyte, which can be found at the top of the quantitation results table below the displayed graph.

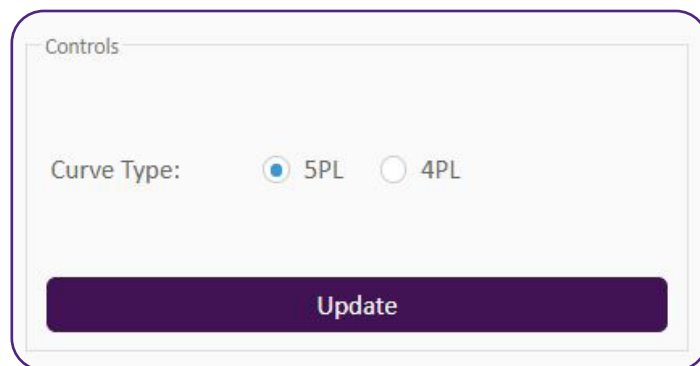
Note: The cell containing the name of the currently displayed graph is shaded a light purple.

The name of the currently selected analyte curve is displayed above the graph. Below the analyte name, the following important information is displayed:

Curve: The curve fitting algorithm used to derive the standard curve is listed here; the default is 5-parameter logistic regression (5PL). Users can also elect to use a 4-parameter logistic regression (4PL) curve fitting algorithm. To switch between the 2 algorithms, first click the cog icon in the upper right of the graph to open the curve fitting controls window (Figure 17).

Note: This same tool icon can be found in each of the analyte name cells at the top of the quantitation results table. Use this tool to toggle between 5PL and 4PL curve fitting on an individual analyte basis.

Figure 17. Curve Fitting Controls



R2: The R2 value, a statistical measure of how closely the regression line fits the standard curve data points, is shown here.

5PL vs. 4PL: In general, the 5PL algorithm provides the best fit regression line to assay data. The software evaluates the standard curve data using both the 5PL and 4PL algorithms, and then performs a statistical test comparing the goodness of fit for each of the regression lines (F-test comparing the residuals at each standard curve point for each algorithm). If the p-value listed here is < 0.05, the 5PL algorithm should be used. If the p-value is non-significant, then either algorithm can be used.

LOD: The limit of detection for the selected analyte. This is the minimum concentration value that can be statistically discriminated from the background signal of the assay blanks (C0).

Standard Curve Plot Display Features

Each standard curve graph contains the following display elements:

Standard Curve Points: All non-zero standard curve points (i.e. C7-C1) are displayed as blue dots on the selected graph.

- Mouse over any data point on the graph to bring up its corresponding PE-MFI and bead count information.

Regression Line: The logistic regression line derived by the curve fitting algorithm is displayed on the graph as a solid blue line.

LOD/LOQ Limits: The theoretical limit of detection (LOD) and limit of quantification (LOQ) are depicted as red and orange vertical lines, respectively.

- Mouse over either of the limit lines to bring up further details.

Other Standard Curve Plot Tools

Move the mouse cursor to the upper right corner of the standard curve graph and a hidden toolbar with 3 icons will become visible. The tools accessible from this bar include the following:

Download Plot: Click this icon to download an image of the currently selected graph.

Zoom: Select this tool and then move the mouse cursor onto the curve plot. Click and drag a box around an area of interest to zoom into the defined space.

Reset Axes: Click this tool to reset the original view of the curve plot.

Quantitation Results Table

The quantitation results table can be used to display the following information for each uploaded FCS data file:

1. Calculated concentrations of assay analytes.
2. Event counts for each Bead ID gate.

3. PE-reporter median fluorescence intensity (MFI).
4. % CV for any defined replicates.
 - Toggle between these views by selecting the desired category from within the 'Color By' circular icon list above the table (Figure 18).

Figure 18. Quantitation Results Table

<input type="checkbox"/>	Sample	Dilution	Replicate	Well	TNF- α (A4)	IL-13 (A5)	IL-4 (A6)	IL-10 (A7)	IL-6 (A8)	IL-2 (A10)	TNF- β (B2)	IFN- γ (B3)	IL-17A (B4)	IL-12p70 (B5)	APRIL (B6)	BAFF (B7)	sCD40L (B9)
	C0		1	C0_1.fcs	<1.40	<1.03	<2.81	<0.60	<3.06	<2.62	<1.06	<0.91	<0.51	<0.77	<0.58	<2.77	<1.31
	C0		2	C0_2.fcs	<1.40	<1.03	<2.81	<0.60	<3.06	<2.62	<1.06	<0.91	<0.51	<0.77	<0.58	<2.77	<1.31
	C1		1	C1_1.fcs	2.48	2.15	4.90	2.92	<3.06	2.78	3.75	3.78	3.37	2.88	2.54	3.17	2.74
	C1		2	C1_2.fcs	2.48	2.83	<2.81	2.26	<3.06	<2.62	2.74	2.70	2.51	2.14	1.68	<2.77	2.26
	C2		1	C2_1.fcs	12.66	11.29	13.66	13.07	14.12	14.62	15.08	12.36	11.50	12.55	16.23	18.29	15.77
	C2		2	C2_2.fcs	8.27	8.76	7.21	7.64	9.40	7.36	8.66	8.83	8.34	8.29	9.47	6.16	7.94
	C3		1	C3_1.fcs	36.94	39.12	32.98	35.65	35.12	37.28	34.78	28.14	33.20	36.42	35.94	35.29	34.42
	C3		2	C3_2.fcs	34.40	35.26	29.82	31.29	30.09	33.20	30.08	27.84	28.51	32.03	31.37	32.61	30.98
	C4		1	C4_1.fcs	181.71	190.08	201.06	208.44	205.35	192.92	168.48	194.83	178.44	215.07	177.47	195.95	192.21
	C4		2	C4_2.fcs	154.62	153.04	159.22	170.67	150.89	174.17	132.76	152.54	153.80	153.19	156.55	150.34	159.17
	C5		1	C5_1.fcs	816.74	656.29	779.85	680.55	765.93	663.19	725.39	1124.16	918.21	751.91	746.59	690.51	762.45
	C5		2	C5_2.fcs	474.75	468.95	500.50	486.84	513.76	399.54	502.50	507.62	550.67	465.77	502.54	468.99	430.68

- To see where a particular experimental sample falls on the currently selected standard curve plot, select the checkbox located to the left of the sample name column. Selected samples will be displayed as orange dots on the graph.

Note: To select all files, check the box located at the top left of the quantitation results table.

Concentration Table

The calculated concentrations for all uploaded FCS files are displayed on this table. Individual values are color coded from blue (low) to red (high) to indicate relative concentration differences.

Select "Show Concentrations outside LOD" to display computed concentration values below the Lower Limit of Detection. Otherwise these values are displayed as <LLOD and are plotted at the x-axis value for the LOD.

Count Table

The count table displays the bead count number for each bead ID gate. All LEGENDplex™ assays recommend collecting 300 events per bead ID in order to ensure robust statistical estimations of PE-report signals.

Bead IDs that contain less than 100 events must be interpreted with caution. Cells that contain less than 100 events are shaded in light yellow. Note: Users can adjust this threshold using the Cell Count Cut-off Slider found in the right corner directly above the table.

MFI Table

The median PE-MFI (Reporter) signal for each gated bead ID population is listed in this table for all uploaded FCS files. Individual values are color coded from blue (low) to red (high) to indicate relative intensity differences.

CV Table

The coefficient of variation, represented as a percentage, is displayed in this table for any FCS files that have been designated as replicates. Replicates with CVs greater than 30% may yield inaccurate quantitation results and should be interpreted with caution. Cells with values > 30% are shaded light yellow.

Note: Users can adjust this threshold using the CV Cut-off Slider found in the right corner directly above the table.

Additional Analysis Tools:

Excluding Data from Analysis

If necessary, users can exclude data from analysis by the software. Users can exclude entire FCS files from analysis as well as single analyte wells within each FCS file. There are 2 methods that can be utilized to accomplish this:

From the Quantitation Results Table

- To exclude an entire FCS file from analysis, locate the file name in the 'Well' column of the table. Right click the name in this cell and select 'Exclude'.
- To exclude a single analyte well from an FCS file, right click the well and select 'Exclude'.

The software will then automatically recalculate the analysis to reflect this exclusion. The analyte wells for the excluded file will be shaded light grey with the values struck through within the cell.

- To undo either action, repeat the above process and click 'Unexclude'.

From the Standard Curve Graph

- Any data point displayed on an analyte standard curve can also be excluded from analysis. Mouse over the desired point and right click to select the 'Exclude' function.

The software will then automatically recalculate the analysis to reflect this exclusion. The analyte well for the file will be shaded light grey with the values struck through within the cell, and the blue circular icon depicting the data point will also be changed to a blue X.

- To undo this action, right click the blue X and click 'Unexclude'.

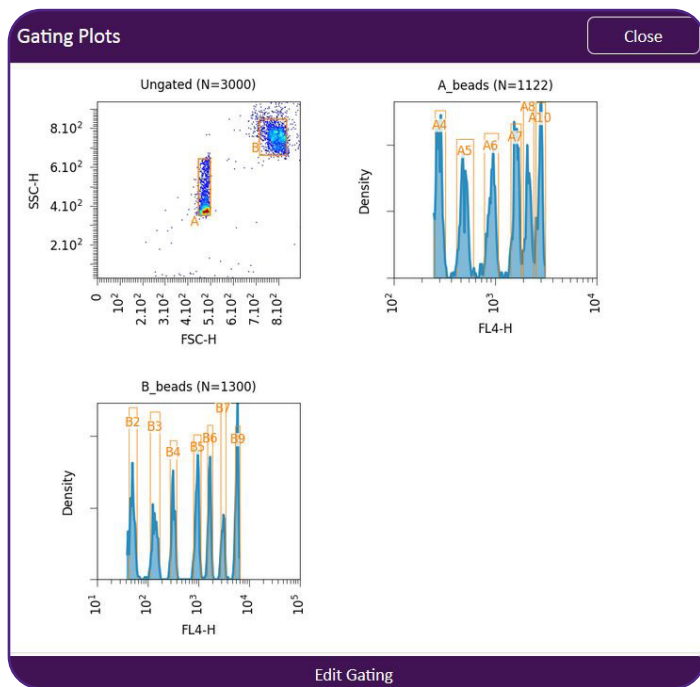
Quick Display of Gating Plots

Users can quickly access a display window of all gating plots for a particular FCS file from the Results Tab.

- Click on any cell in the row associated with the desired FCS file in the quantitation results table to access the Gating Plots Window (Figure 19).

Note: This can also be accomplished by clicking on any data point displayed on a standard curve graph.

Figure 19. Gating Plots Window



- Click the purple 'Edit Gating' button at the bottom of the window if gating adjustments are required.

This will return users to the Gating Review Tab where gates can be adjusted using the method discussed in the 'Adjusting Gates' section of this manual.

Generating Data Reports:

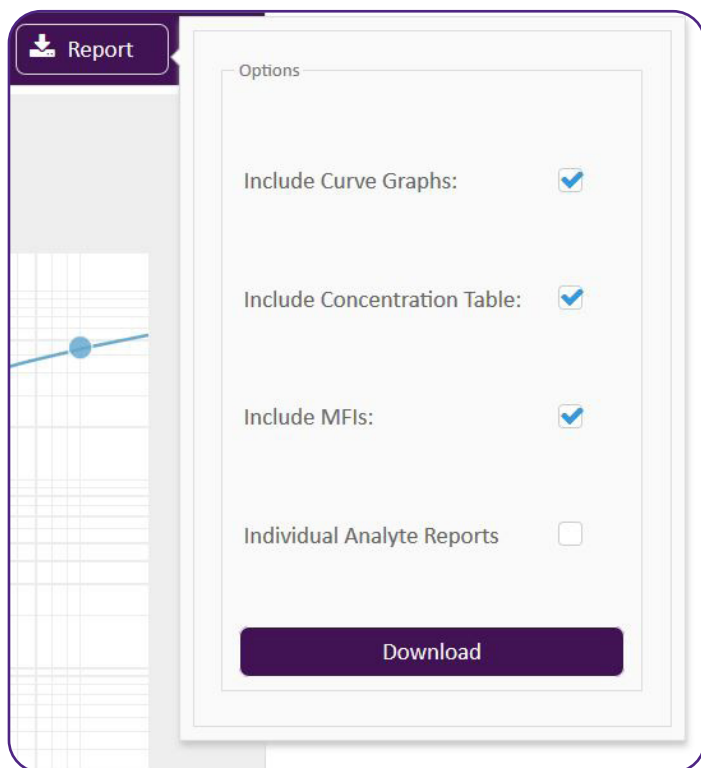
The quantitation results can be readily downloaded into an Excel file which summarizes all relevant assay information. Users can select which parameters they would like to include in the report.

By default, the standard curves, concentration table, and MFI (PE-reporter) signals are selected, but can be deselected. Additionally, the individual analyte reports box can be checked, which will add a separate summary tab for each individual analyte in the assay.

To download a Data Report, complete the following:

- Click the 'Report' button located at the top right of the standard curve graph (Figure 20).
- Check the boxes for all desired options.
- Click the 'Download' button at the bottom of the options window.

Figure 20. Report Button



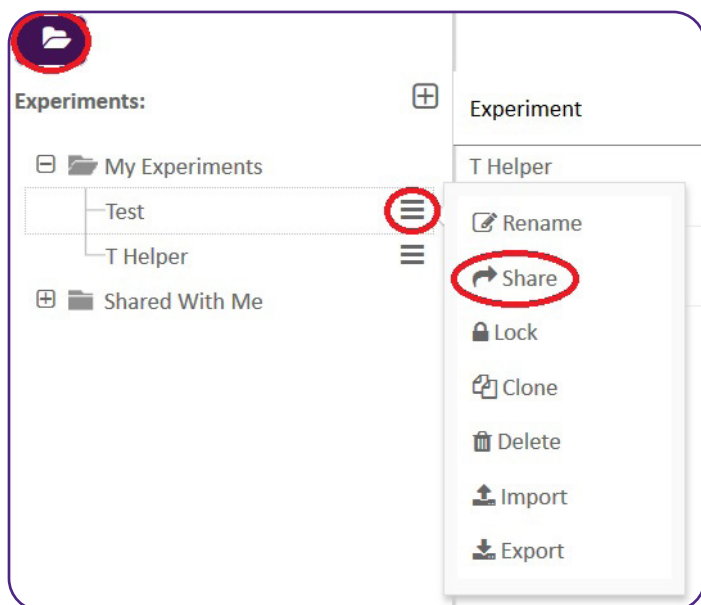
Data Management & Storage:

Sharing Experiments (Cloud Software Only)

The software allows you to share experiments with other registered users. Once shared, the other party can review and edit your experimental data – making collaboration a matter of a few mouse clicks.

Note: If desired, editing privileges can be disabled by locking the experiment prior to sharing. This will allow collaborators to view the shared workspace without being able to modify the results.

Figure 21. Folder Icon Menu & Data Sharing



To share a particular experiment, complete the steps highlighted in Figure 21:

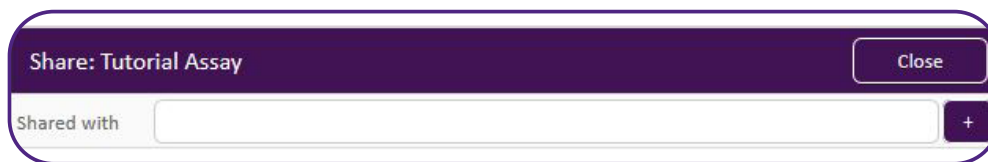
- Click the Folder Icon in the upper left of the screen.

- Navigate to the experiment in the 'My Experiments' list.
- Click the Menu button to the right of the experiment name.
- (Optional) Select 'Lock' to disable editing of the experiment (repeat this process to Unlock).
- Select 'Share' from the menu options to open the Sharing Window (Figure 22).
- Enter the email address of a registered user in the 'shared with' field.

The software will instantly share the data with the designated collaborator account, with the experiment now appearing in their 'Shared with Me' files list in the Folder Icon Menu (Figure 21). Likewise, any experiments shared with your account can be found listed here as well.

Note: To share an experiment with a collaborator, you must enter the email address used as their account name. In order to respect privacy considerations, the software does not contain a searchable user-list. The only exception to this case is BioLegend Technical Service, which is immediately discoverable upon clicking in the 'Shared With' field.

Figure 22. Sharing Window



Cloning Experiments (Cloud Software Only)

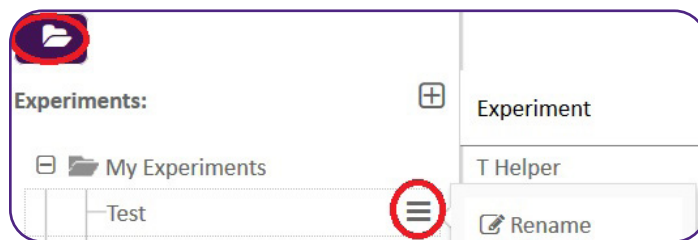
The software allows users to clone uploaded experiments. This process creates a duplicate experiment, which is renamed but retains exactly the same data and analysis settings.

This feature may be of utility for users who wish to share an editable experiment with a collaborator, but would like to retain the original analysis as well. In this case, an unlocked experiment clone could be created and shared while the original remains unshared.

To clone an experiment, please complete the steps highlighted in Figure 23:

- Click the Folder Icon in the upper left of the screen.
- Navigate to the experiment in the 'My Experiments' list.
- Click the Menu button to the right of the experiment name.
- Select 'Clone' from the menu list to access the 'Clone Window'.
- Enter a new name for the cloned assay in the 'Experiment Name' field.
- Click 'Clone' button at the bottom of the 'Clone Window'.

Figure 23. Cloning Experiments



Export/Import of Experiments

Should users need to free up storage space on their Cloud software account, entire experiment workspaces can be downloaded and saved as a compressed file (.lpx extension) that can be easily reimported into the software at a future date. After exporting an experimental workspace, the assay results can be deleted from the Cloud software server.

The compressed file contains all relevant FCS file data and analyses, and can be reloaded using the software's import function to easily access the analysis again.

Experiment workspaces exported from one program may be imported into all other programs within the LEGENDplex™ Data Analysis Software Suite.

To export an experiment, please complete the following steps:

- Click the Folder Icon in the upper left of the screen.
- Navigate to the experiment in the 'My Experiments' list.
- Click the Menu button to the right of the experiment name.
- Select 'Export' from the menu list.
- Follow the Software's instructions.
- Save the compressed folder (.lpx) to your local hard drive.

To import an experiment, please complete the following steps:

- Create a new experiment.
- Enter the desired name in the 'Experiment Name' field.
- Click 'Create+'.
- Click the Folder Icon in the upper left of the screen.
- Navigate to the experiment in the 'My Experiments' list.
- Click the Menu button to the right of the experiment name.
- Select 'Import' from the menu list.
- Click the 'Select .lpx File' button.
- Navigate to the location of the exported .lpx folder on your hard drive.
- Click 'Open'.
- Click the 'Import' button at the bottom of the import window.

Contact BioLegend

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