Second Edition February 2006

LiquiChip® Analyzer Software Basic Users Guide

For analysis of data generated using xMAP™ instruments

QIAGEN

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Introduction

This handbook is intended as a short, basic introduction to the functions and features of LiquiChip® Analyzer software. It is divided into two sections. The initial section (pages 4–15) gives a brief introduction to the features of the software and enables the user to load, analyze, and export data generated using a LiquiChip Reader or similar xMAP® instrument.

Advanced features of the software are addressed in the second section (pages 16–26).

The LiquiChip Analyzer Software enables fast analysis and quantification of LiquiChip xMAP assays. Raw data is input as an output.csv file. Easy-to-use and intuitive environments let researchers define plate locations of standards, controls, and unknowns. Once information on standard concentrations is entered, concentrations of analytes are calculated using either a user-defined or best curve fit algorithm. Results can be exported in tabular or graph form.

Generating Raw Data Using Luminex IS 2.3. Software

Creating and running an advanced batch is described in the *IS 2.3 Software User Manual 03/2006*. Data are saved exclusively in the output.csv and run file and can be further analyzed or examined using the IS 2.3 Software.

Creating a template for assays that give raw data only is described on page 5-137 of the *QIAGEN LiquiChip Workstation User Manual*.

Creating a batch is described on page 5-62 of the *QIAGEN LiquiChip Workstation User Manual*.

To generate raw data suitable for analysis using LiquiChip Analyzer Software:

- The “Enable Raw Data Storage” box must be checked in the “General” tab of the “Options” dialog box.
- The “Trimmed %CV” box must be checked in the “Data Export” tab of the “Options” dialog box.

Screen Architecture

LiquiChip Analyzer software is based on familiar Windows® type architecture. Figure 1 shows the typical architecture of the LiquiChip Analyzer Software screen.

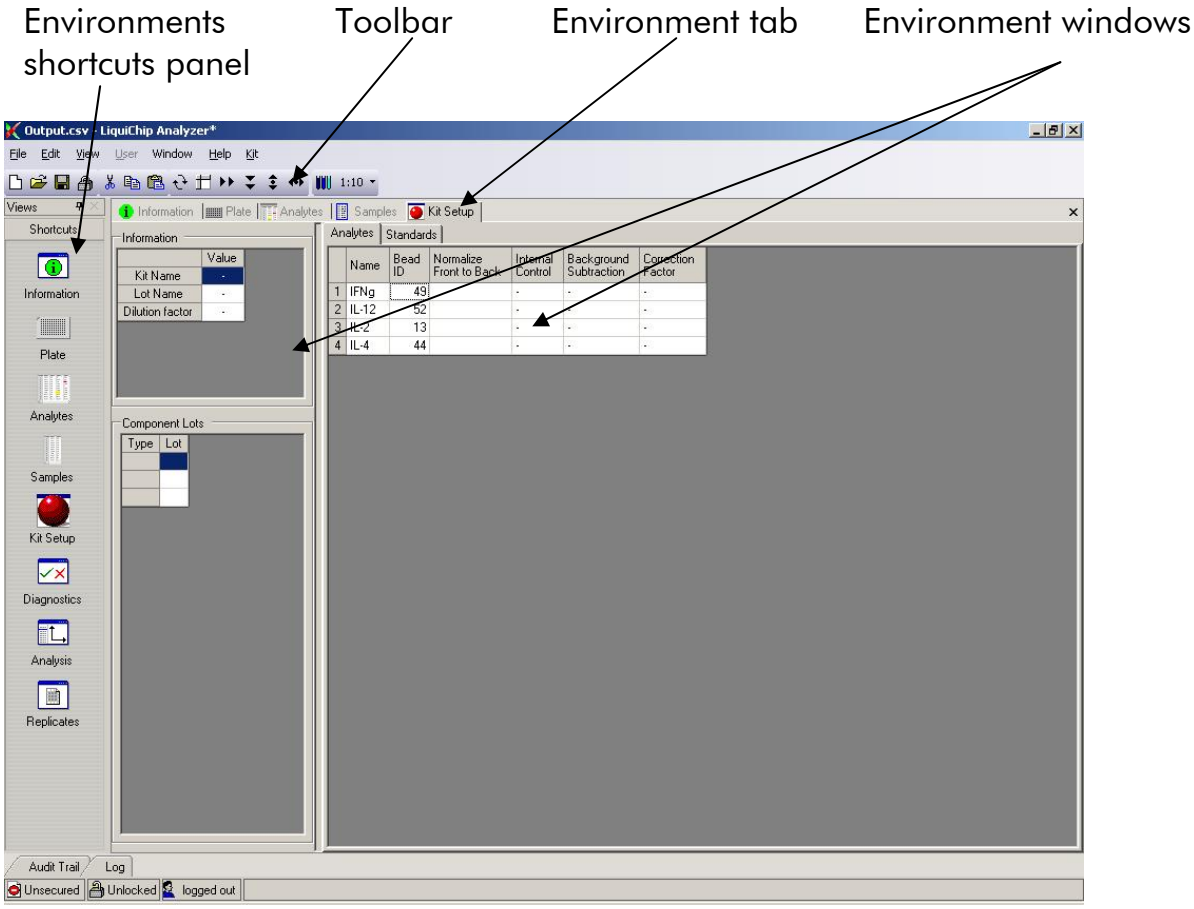


Figure 1 LiquiChip Analyzer Software screen architecture.

LiquiChip Analyzer Software Environments

The LiquiChip Analyzer Software contains eight separate environments. They are accessed by clicking on the relevant icon in the Environments shortcuts panel (see Figure 1). Once opened, each environment can also be accessed by clicking on the relevant Environment tab below the toolbar or by choosing the relevant environment from the "Window" drop-down menu.

Information environment

This environment contains information about the run generated by the output.csv file. This information includes machine serial number, application, and plate name. Comments can be added or deleted using the "Add Comment" or "Delete Comments" buttons.

Plate environment

This environment shows information about the samples on the plate being analyzed. Predefined information includes the sample name (if entered in IS 2.3 software) and total number of beads analyzed per well. Sample information that must be added by the user before data analysis includes the type of sample (e.g., background, control, or standard) and sample dilution factor. A breakdown of the data can also be displayed for each analyte. Groups of replicate samples can also be defined in this environment.

Analytes environment

This environment displays in tabular form both raw data (e.g., bead count and raw MFI) and values calculated after analysis. A breakdown of the data is displayed for each analyte.

Samples environment

This environment displays the same data and information that is displayed in the "Plates" environment in tabular form. Sample and replicate information can also be directly entered in this environment.

Kit Setup environment

The Kit Setup environment enables users to define parameters for the calculation of the concentration of each analyte (e.g., whether or not to carry out a subtraction of background or to define a particular analyte as an internal control). The concentration of each standard and limits for acceptable data values are also defined on this environment.

Diagnostics environment

The "Diagnostics" environment allows users to apply diagnostics algorithms to their data in order to filter out inconsistent or low-quality data.

Analysis environment

The "Analysis" environment performs regression analysis on the standard curves and generates results for the unknown samples and a graphical representation of standard curves.

Replicates environment

The "Replicates" environment displays average results, %CV, and SD values for identical samples defined as groups in the "Plate" or "Samples" environments.

Analyzing xMAP Data Using LiquiChip Analyzer Software

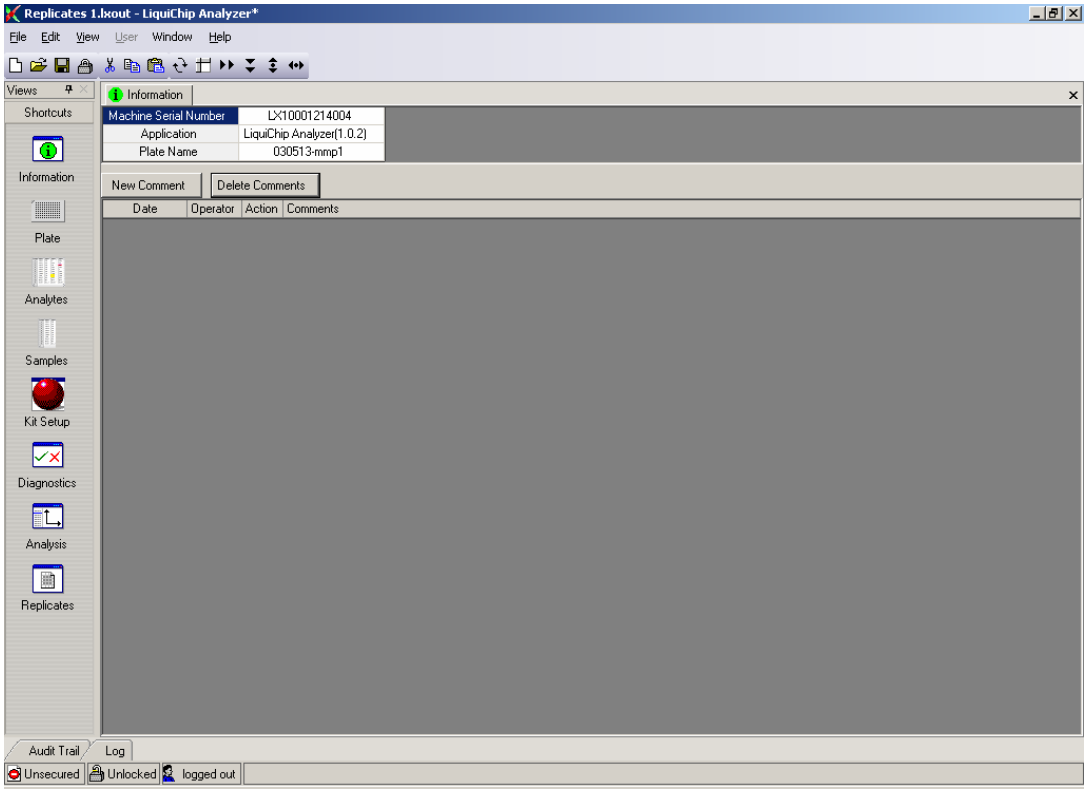
The process of analyzing xMAP data can be broken down into 8 steps.

- Loading raw xMAP data (see page 9)
- Defining sample types (see page 10)
- Entering standard concentrations (see page 14)
- Performing a diagnostics data check (see page 21)
- Generating standard curves (see page 14)
- Analyzing raw data using the parameters entered in the first three steps (see page 15)
- Analysis of sample replicates (see page 25)
- Export or printing of data (see page 26)

Opening and Loading Raw xMAP Data Files Using LiquiChip Analyzer Software

1. **Launch the LiquiChip Analyzer Software by clicking on the icon on the desktop. The "Information" environment is automatically opened.**
2. **Open the output.csv file using the File > Open command or the icon on the toolbar.**

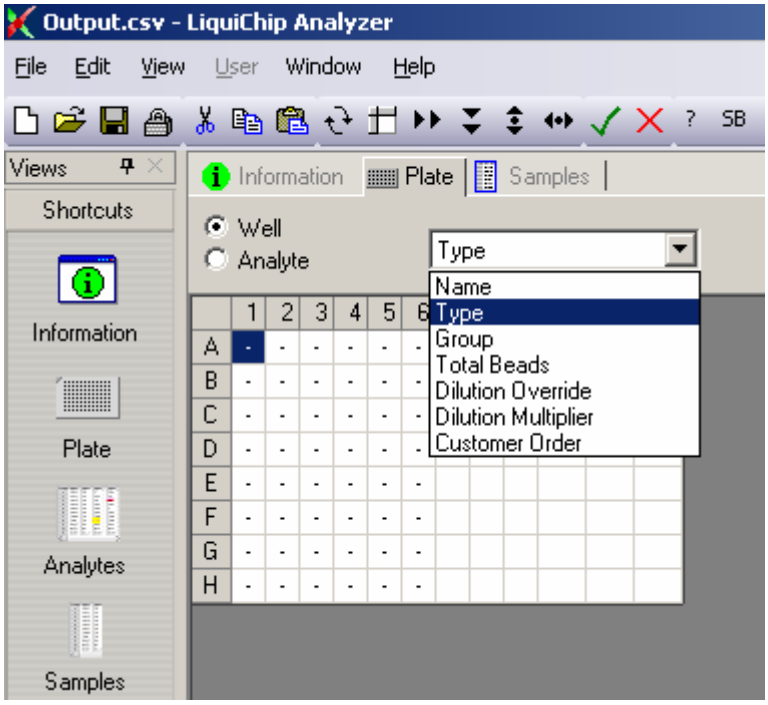
   Machine serial number, application, and plate name are displayed.



Defining Sample Types

Defining the location and type of sample on a plate is performed in the "Type" window or column of the "Plate" or "Samples" environment respectively. In the "Plate" environment the sample information is displayed in a 96-well plate format, and in the "Samples" environment in a table format (see below). To enter sample type information in the "Plate" environment click on the "Well" radio button and choose "Type" from the drop-down menu (see below).

Tip: When working in the "Plate" environment, highlight and copy the information in the "Well" > "Name" fields. Select "Type" from the drop-down menu, and paste the information into the corresponding wells. Overwrite the "Name" fields with the "Type" information. This saves having to switch between "Name" and Type" fields while defining sample types. Information in the "Name" and "Total Beads" fields is imported from the output.csv file.



"Plate" environment

Entering sample information

The location and type of each sample is entered by highlighting the relevant well(s) ("Plate" environment) or cell(s) ("Samples" environment) and clicking on the corresponding button in the toolbar, or by copying and pasting defined wells or cells.

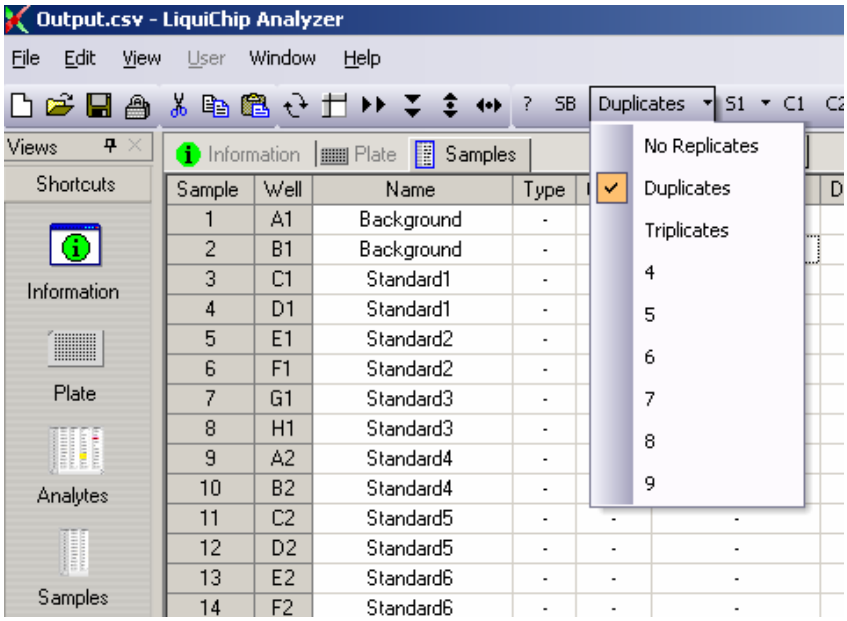
There are four types of sample that can be defined:

- **Unknown Sample (? on the toolbar)** — wells containing test samples whose concentration is calculated on the basis of the standard curve defined using the selectable standards
- **Sample background (SB on the toolbar)** — "blank" wells. The MFI value in wells defined as SB can be subtracted from test values to normalize data.
- **Selectable standards (S1–S^n on the toolbar)** — wells containing the dilutions of standards which make up the standard curve. Define the most dilute standard as S1, the next most dilute as S2, and so on.
- **Controls (C1=1st level control, C2 = 2nd level control, etc.)** — wells that contain "internal controls" that serve as reference values for unknown samples.

Automatically defining and entering multiple standard sample types

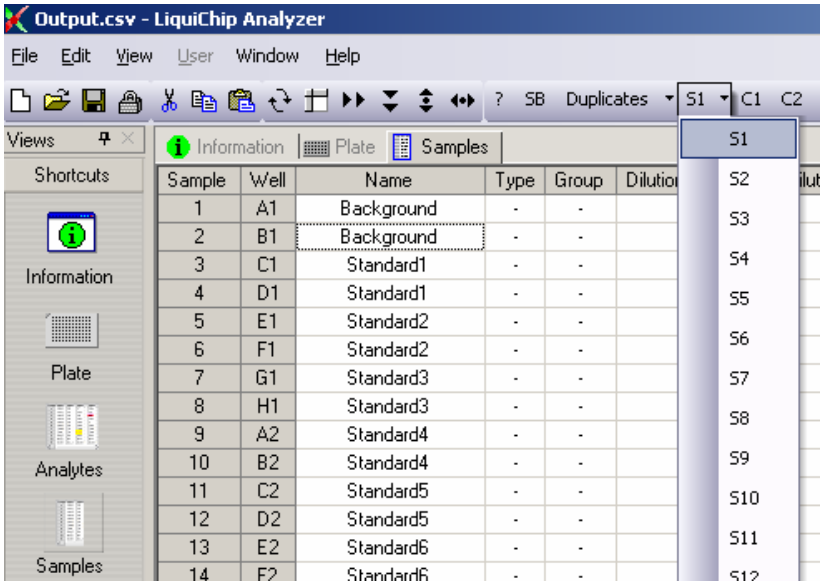
LiquiChip Analyzer Software allows multiple standard sample types to be defined and entered in the "Plate" or "Samples" environments. The example shown below is performed in the "Samples" environment.

1. **Define the multiplicity of the wells containing standard-curve samples using the drop-down menu on the toolbar.**

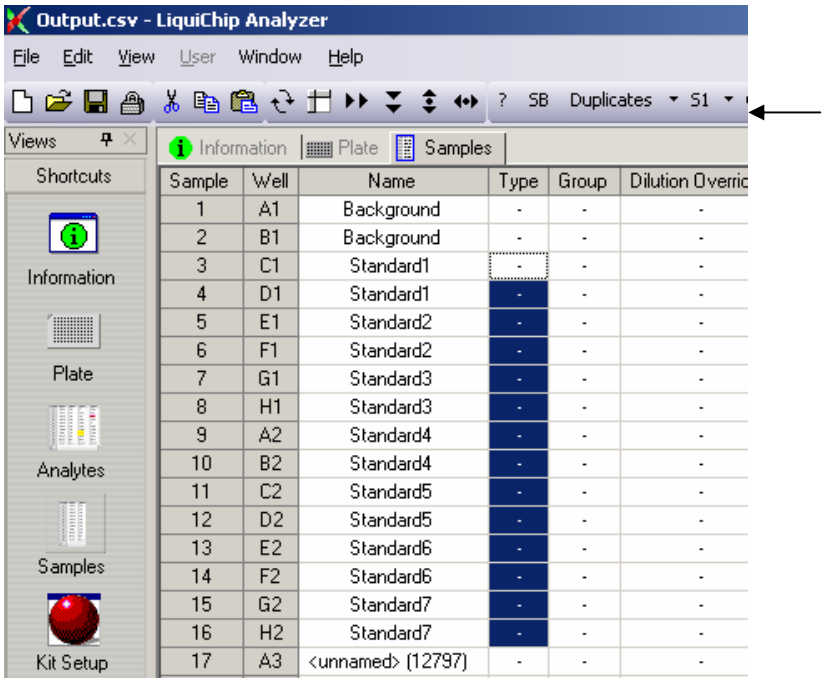


2. **Select the standard level with which you wish to begin the series (usually S1) in the "Selectable standard" drop-down menu in the toolbar.**

   Note: The standard levels define each concentration in a dilution series. For example, when using a standard curve containing 8 duplicate points from blank to most concentrated, define the two blank wells as SB, the most dilute standard wells as S1, the next most concentrated as S2, and so on until S7, the most concentrated (see below).



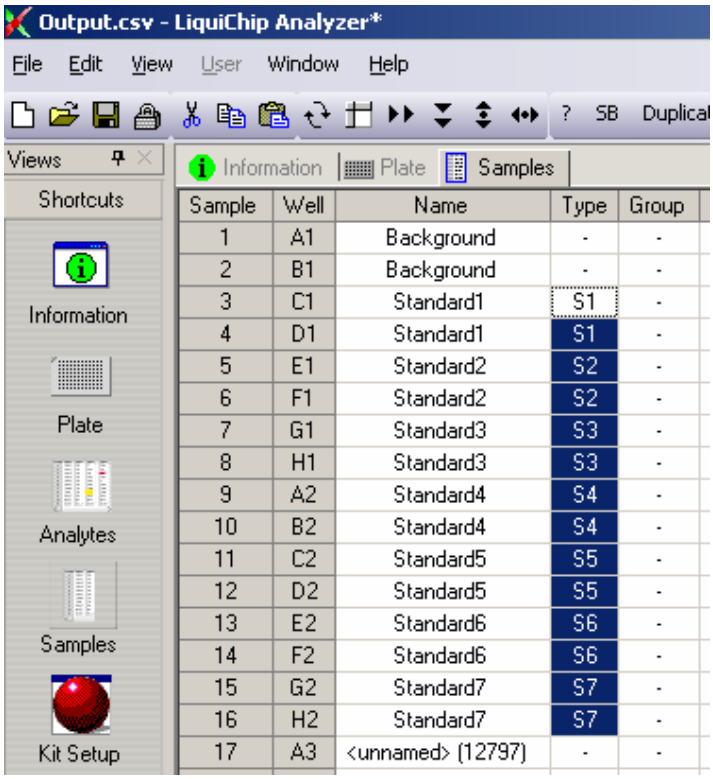
3. **In the "Type" column, highlight the cells corresponding to the standard wells.**



"Selectable standard" button

4. **Click on the "Selectable standard" button in the toolbar.**

   The software automatically defines the highlighted wells as standard wells with the desired multiplicity.



Generating average analyte values from identical samples

Average analyte values for multiple identical samples can be obtained by defining the relevant samples as groups. An average analyte result, %CV, and SD are displayed in the "Replicates" environment for all wells that are assigned an identical identifier in the "Group" window ("Plate" environment) or column ("Samples" environment) (see page 25).

Entering Standard Concentrations

Concentrations of standards that make up the standard curve(s) are entered in the “Standards” tab of the “Kit Setup” environment.

Manual entry

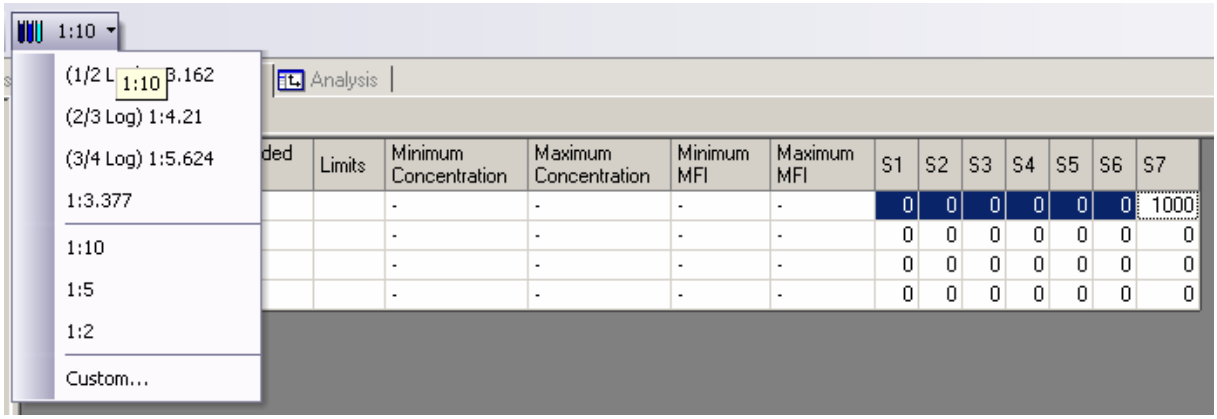
Values can be entered directly into the relevant cells, or copied and pasted from spreadsheets.

Automatic calculation of dilution concentrations

LiquiChip Analyzer software can automatically calculate and enter standard serial dilution concentrations.

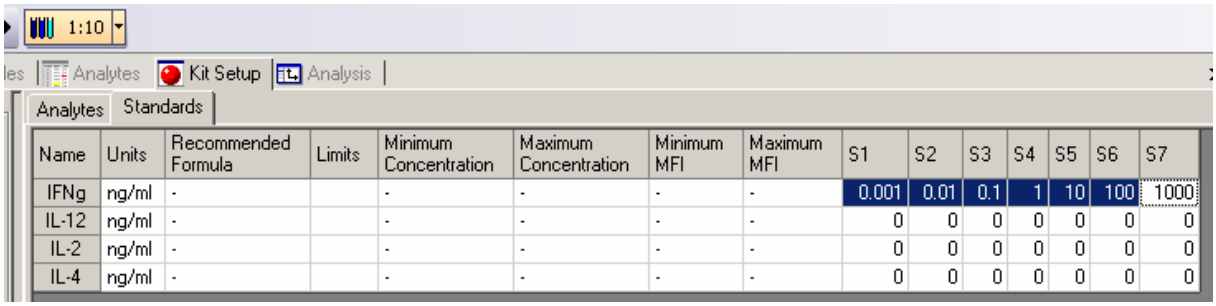
1. **Enter the concentration of the most concentrated standard (e.g., S7 for an 7-point standard curve).**
2. **Highlight all the cells that correspond to the standard serial dilutions (including the most concentrated standard).**
3. **Select the relevant dilution factor from the drop-down menu in the toolbar or enter a custom value.**

   The dilution factor will appear in the toolbar button.



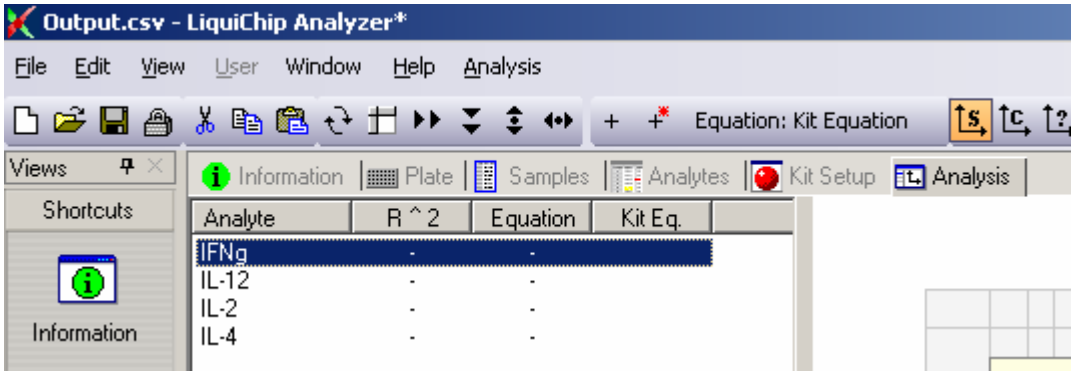
4. **Click on the dilution button in the toolbar.**

   The concentration of each dilution is automatically calculated and entered into the relevant cell.



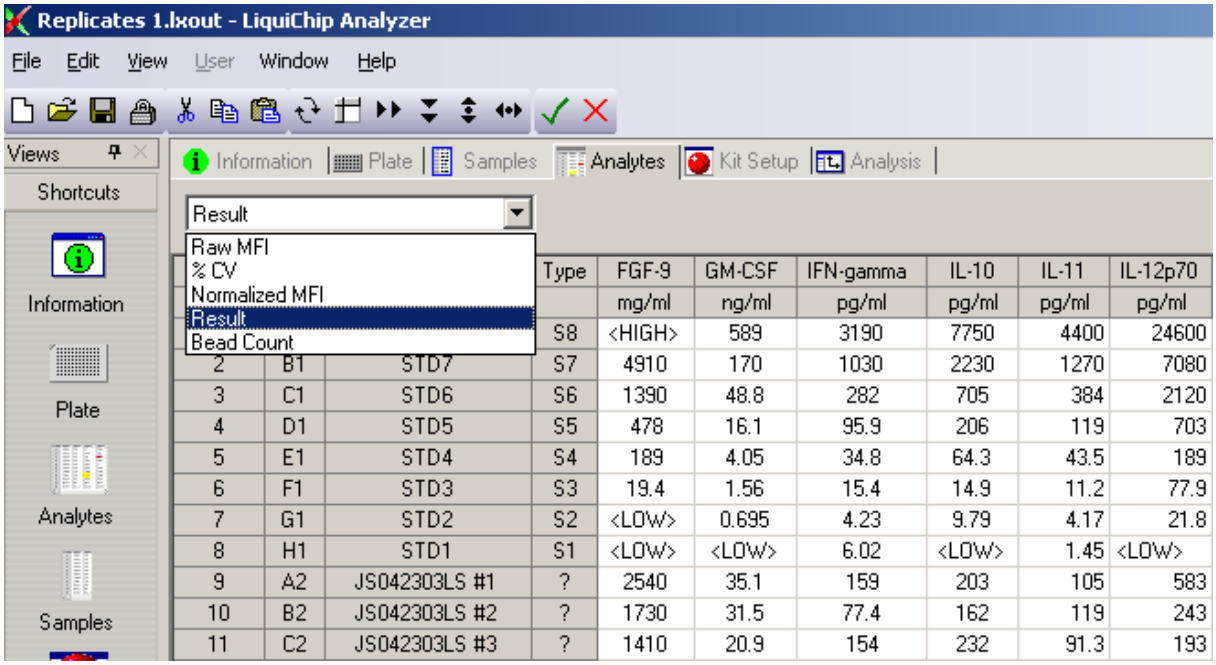
Analyzing Raw Data

Once raw data has been loaded, well types have been defined, and standard concentrations have been entered, standard curves and the analyte concentration in unknown samples can be calculated. This analysis is performed in the “Analysis” environment.



Standard curves and calculation of the concentration of unknowns is performed by clicking the either the + “Calculate Selected Analyte” or +* (“Calculate All Analytes”) button on the toolbar.

Calculated concentrations of unknown samples can be viewed in the “Result” field of the “Analytes” environment.



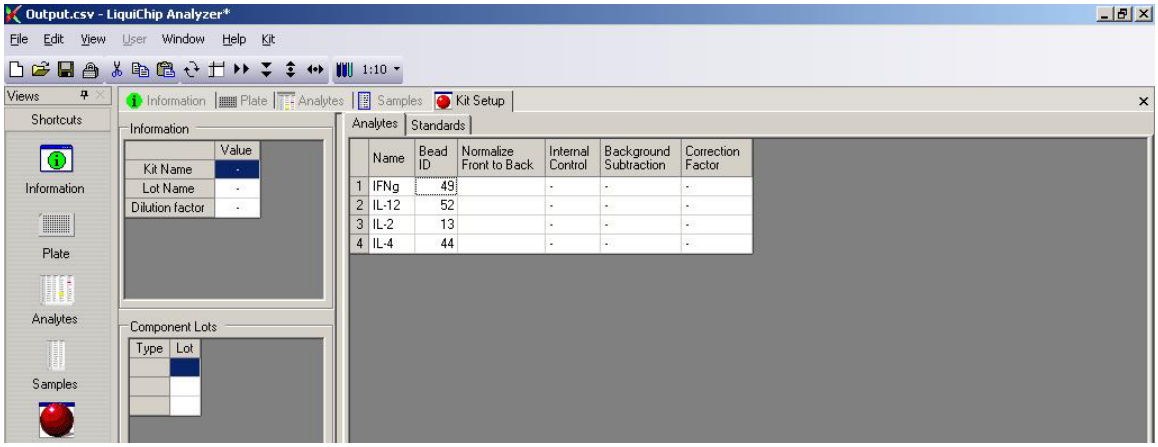
Advanced Features

“Plate” and “Samples” environments

There are further parameters that can be defined in the “Well” screen of the “Plate” environment and “Samples” environment:

- Group — this function is used to define groups of identical samples. The identifier for each group is a group name. After analysis, the software averages the results for all the wells in a group to give a mean analyte value, %CV, and SD.
- Dilution Override — any dilution factor defined here will be used to calculate the concentration of a sample, irrespective of any other dilution information entered in other locations (see below)
- Dilution Multiplier — this function is used to modify any dilution factor applied across the entire plate (defined in the “Kit Setup” environment). For example, if the “Dilution factor” defined in the “Kit Setup” environment is 5, applying a “Dilution Multiplier” of 10 to a well will result in a total dilution factor of 50 being applied.

“Kit Setup” environment



The “Information” window of the “Kit Setup” environment contains fields in which “Kit Name”, “Lot Name”, and “Dilution factor” information can be entered.

The “Component Lots” window can be used to record lot information on the reagents used in the assay.

The large window in the “Kit Setup” environment displays either the “Analytes” or “Standards” window, depending on the chosen tab.

“Analytes” window

The following parameters can be defined in the “Analytes” window of the “Kit Setup” environment.

- Name — names of analytes are imported from the output .csv file.
- Bead ID — bead code information, imported from the output .csv file. **This information cannot be altered.** If the bead-code information were changed, analysis would not be possible.
  Note: If data are imported from IS 2.x software, the bead codes are automatically reassigned a new value from –1 to –x. If you wish to import the data in a particular order open the following tab in the IS Software Tools>Options>Data Export. In the Test Sort Order field click on
  Alphabetical by Test Name — to export data arranged alphabetically according to the test name
  Sequential by Region ID — to export data arranged according to increasing bead code
  Template Setup Order — to export data arranged according to the order of template setup

- Normalize Front to Back
  - None — default value.
  - Front-back standards — choosing this option applies an algorithm to the assay results that takes the “plate effect” into account when calculating final concentrations. This feature can only be used when two identical standard curve dilutions are present in the first and last columns of an assay.
- Internal Control
  - Yes — choosing this option enables an analyte to be defined as an internal control.
  - No — default value.
- Background Subtraction
  - Yes — Default value. Choosing this option subtracts the average value in wells defined as “Sample Background” in the “Plate” or “Samples” environments (see page 10) from raw MFI values. These values can be displayed by choosing “Normalized MFI” in the drop-down menu in the “Analyte” screen of the “Plate” environment or in the “Analytes” environment.
  - No — shows raw MFI values.
- Correction Factor — any multiplication factor entered here will be used in calculating the final result. This feature is useful for correlating data to given external standards (e.g., WHO standards).

“Standards” window

The following parameters can be defined in the “Standards” window of the “Kit Setup” environment.

- Units — the units used to define concentrations (e.g., pg/ml) can be defined here.
- Recommended Formula — drop-down menu allowing choice of analysis algorithm.
  - 4PL and 5PL — Logistic 4- or 5-point fitting.
  - 4PL (Log) and 5PL (Log) — Logistic 4- or 5-point fitting using logs of the raw data.
  - External — imported curve-fitting algorithm.
  - Best fit — default value which applies all the above algorithms to the data until the best fit is found.
- Limits — sets absolute limits on MFI or concentration values below or above which they will be rejected.
  - Relative — Default value. Uses the minimum and maximum standard MFI values to reject data lying outside these boundaries.
  - None — All data values will be accepted.
  - Fixed — uses fixed MFI or concentration values to reject data.
  - External — uses imported limits to accept or reject data.
- Minimum Concentration — minimum concentration below which calculated concentrations will be rejected. The value entered will be applied as a fixed or relative value (see above). For example, if the chosen units are ng/ml and “Fixed” is chosen in the “Limits” field, entering 20 will reject all values below 20 ng/ml. If “Relative” is chosen in the “Limits” field, entering 0.2 will reject all values below 20% of the minimum standard concentration.
- Maximum Concentration — maximum concentration above which calculated concentrations will be rejected. The value entered will be applied as a fixed or relative value (see above). For example, if the chosen units are ng/ml and “Fixed” is chosen in the “Limits” field, entering 200 will reject all values above 200 ng/ml. If “Relative” is chosen in the “Limits” field, entering 0.9 will reject all values above 90% of the maximum standard concentration.

- Minimum MFI — minimum MFI below which data will be rejected. The value entered will be applied as a fixed or relative value (see above). For example, if “Fixed” is chosen in the “Limits” field, entering 20 will reject all MFI values below 20. If “Relative” is chosen in the “Limits” field, entering 0.2 will reject all values below 20% of the maximum standard MFI.
- Maximum MFI — maximum MFI above which data will be rejected. The value entered will be applied as a fixed or relative value (see above). For example, if “Fixed” is chosen in the “Limits” field, entering 20,000 will reject all MFI values above 20,000. If “Relative” is chosen in the “Limits” field, entering 0.9 will reject all values above 90% of the maximum standard MFI.

“Diagnostics” environment

This environment allows users to apply diagnostics algorithms to their data in order to filter out inconsistent or low-quality data. The “Diagnostics” environment window contains 5 diagnostics algorithms which can be toggled by clicking on the relevant algorithm. Active algorithms are indicated by a checked box. Clicking on the “Run” button starts the diagnostics check.

- Low Bead Count – absolute — this algorithm checks the absolute number of beads in each well and generates a warning for wells in which the number of beads is below a defined minimum value.
- Low Bead Count – relative — this algorithm checks the number of beads in each well. It generates a warning for wells in which the number of beads is more than 2 standard deviations below the median value.
- Hook effect — this algorithm checks for saturation effects in standard curves containing high concentrations of analyte in a non-wash or non-filtered assay.
- Internal control — this algorithm detects the presence of an internal control bead and generates a warning for all wells in which it’s MFI value varies by more than 2 standard deviations.
- High RP1 CV – relative — this algorithm generates a warning for all unknown sample wells in which the CV value is above a certain limit, relative to the analyte median CV values across the entire plate. Standards and controls are not checked.

“Analysis” environment

In this environment, data are analyzed on the basis of information entered or imported into the “Samples”, “Analytes”, and “Kit Setup” environments.

Standard curves and calculation of the concentration of unknowns is performed by clicking the either the “Calculate Selected Analyte” or (“Calculate All Analytes”) button on the toolbar.

The equation used to fit the standard curve(s) can be defined by selecting the desired equation from the “Equation:” drop-down menu and clicking the “Calculate Selected Analyte” or “Calculate All Analytes” button on the toolbar.

After a standard curve has been calculated, standards, controls, and unknowns can be displayed on or removed from the curve by toggling the (“Standards”) (“Controls”), and (“Unknowns”) buttons respectively. The buttons will appear orange when selected.

The (“View Previous Curve”) button allows comparison of curves generated by different fitting equations.

The (“Zoom”) button permits chosen section of the curve to be magnified.

Using the drop-down menu on the (“Axis”) button allows linear or logarithmic scales to be chosen for the x- and y-axes.

Right-clicking on the graph opens a dialog box that allows display and export parameters to be defined. The graph can also be exported using the “Export”>”Graph” command in the “File” drop-down menu.

Standard points

Point information (status, well location, raw and normalized MFI) can be viewed by clicking on the (“Information”) button and clicking on the standard point. This button is orange when active and gray when inactive.

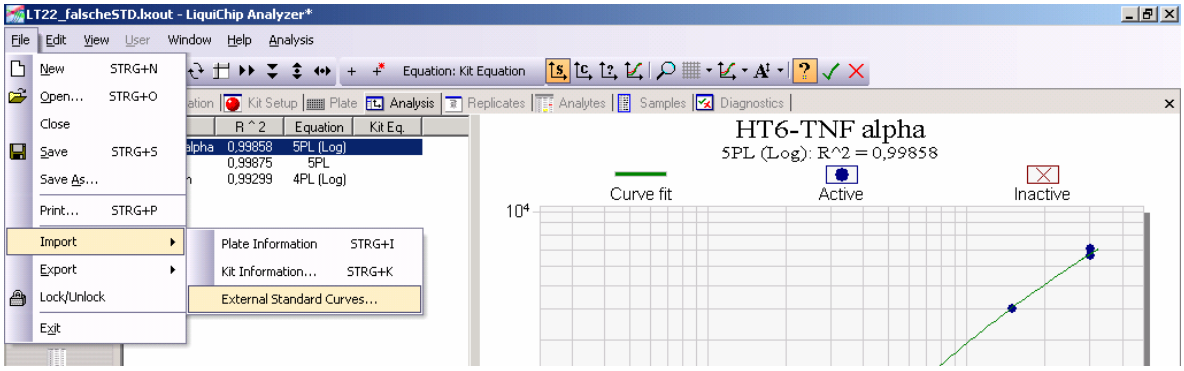
Standard points can be discarded from the fitting of a standard curve by clicking on the (“Reject Standard”) button and clicking on the point to be rejected. This button is orange when active and gray when inactive. The standard curve can then be recalculated using the “Calculate Selected Analyte” button.

Rejected standard points can be included the fitting of a standard curve by clicking on the (“Accept Standard”) button and clicking on the point to be included. This button is orange when active and gray when inactive. The standard curve can then be recalculated using the “Calculate Selected Analyte” button.

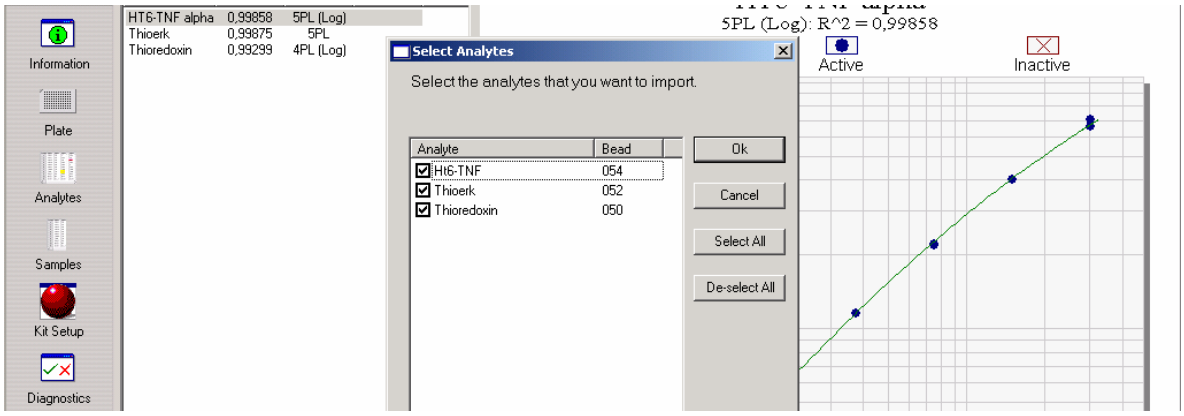
Importing standard curves

Data can be analyzed using an existing standard curve. To import the standard curve, select “External Standard Curves” from the “Import” command in the “File” drop-down menu. Select and open the **.lxout** file from which the standard curve is to be imported. Choose the standard curve(s) to be imported from the list in the dialog box. To analyze data using an imported standard curve, select “External” from the drop-down menu on the “Equation:” toolbar button. The analyte name in the data to be analyzed and the imported standard curve must be identical. Importing a standard curve and using its data for analysis is only possible if it was obtained using the same kit set up as the new assay.

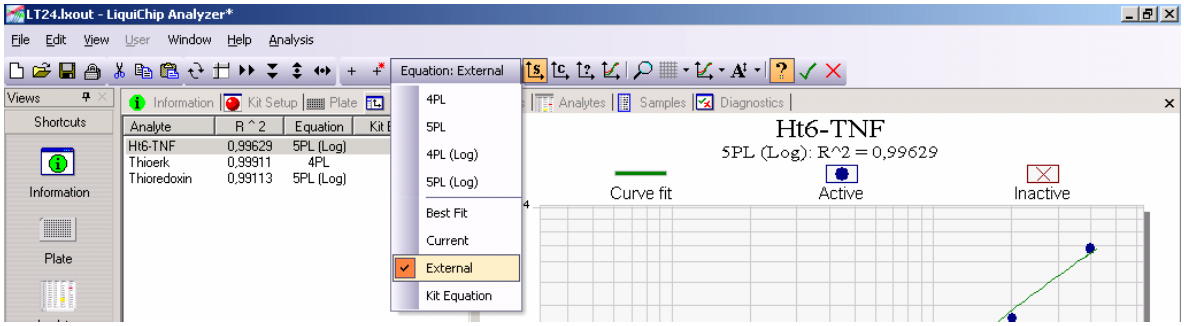
1. **Import the external standard curve data by selecting File>Import>External Standard Curves... .**



2. **Select and open the .lxout file from which the standard curve is to be imported. Choose the standard curve(s) to be imported from the list in the dialog box.**



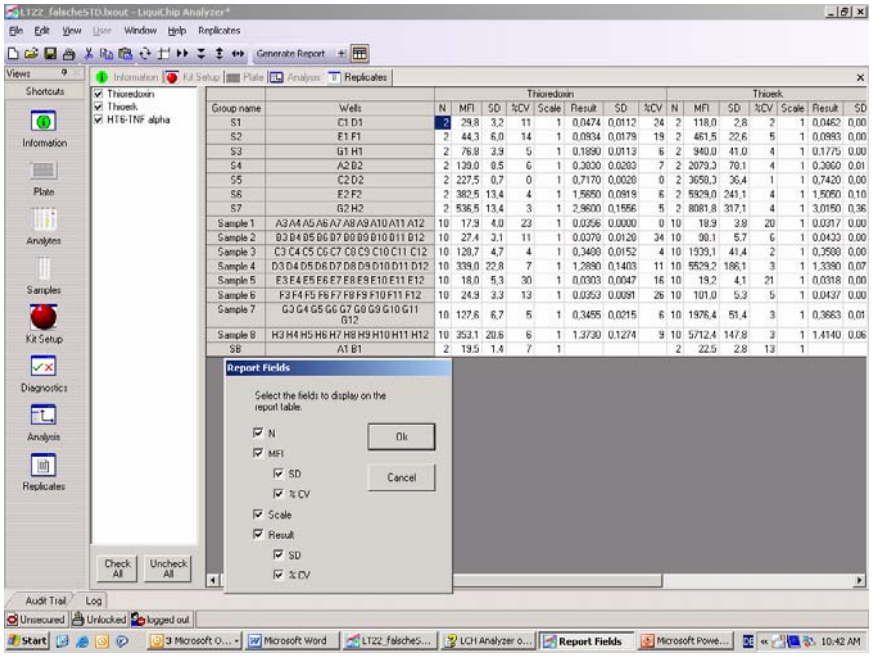
3. **Choose the option “External” in the drop-down menu of the Equation: External toolbar.**



4. **Recalculate the appropriate standard curve(s).**

“Replicates” environment

After analysis of data the “Replicates” environment enables a report on the “Groups” defined in the “Plate” or “Samples” environment to be generated. A basic report, giving the average value for each group is generated by clicking on the “Generate Report” button in the toolbar. The basic report displays the name of the group, in which wells the samples belonging to the group were present, and the average MFI and unit values for each checked analyte.



To choose the fields that you wish to display click on the (Fields…) icon in the Generate Report toolbar and check the relevant boxes.

N — number of replicates

MFI — mean of normalized replicate MFIs

SD — standard deviation of normalized replicate MFIs

% CV — % standard deviation of normalized replicate MFIs

Scale — dilution factor deriving from Dilution Override or Dilution Multiplier (see page16)

Result — mean of calculated replicate results

SD — standard deviation of calculated replicate results

% CV — % standard deviation of calculated replicate results

A more detailed report displaying MFI and unit values for each well in the group can be generated by clicking on the (“Show Details”) button next to the “Generate Report” button in the toolbar.

Exporting and Importing Data

All values and definitions in the “Well” screen of the “Plate” environment and “Samples” environment (“Plate Information”) and in the “Kit Setup” environment (“Kit Information”) can be imported or exported using the “Import” and “Export” commands in the “File” drop-down menu. “Plate Information” is stored as a Plate template (**.lxfmt**) file. “Kit Information” is stored as a Kit template (**.lxkit**) file.

Analyzed data can be saved using the “Save” or “Save As” commands in the “File” drop-down menu. Data is saved as an Output (**.lxout**) file.

Handling data

Information in any data field can be imported from or exported to other spreadsheet-type programs (e.g., Microsoft® Excel). Information in grayed-out cells can be accessed or blocked by toggling the (“Show/Hide grid headers”) button on the toolbar. Other buttons in the toolbar are useful for manipulating data in cells.

Replicate Cells Right — copies the value(s) in the first column of a highlighted block of cells into all highlighted cells to the right of the first column.

Replicate Cells Downward — copies the value(s) in the top row of a highlighted block of cells into all highlighted rows below.

Swap Cells Vertically — flips values in a highlighted block of cells vertically about the central axis.

Swap Cells Horizontally — flips values in a highlighted block of cells horizontally about the central axis.

QIAGEN Companies

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