Getting Good Data with xPONENT®

Software for the Luminex® MAGPIX®, Luminex® 100/200™, and FLEXMAP 3D® Systems

FLEXMAP 3D®

MAGPIX®

Luminex® 100/200™

xPONENT® is a modular, flexible software package for control of the MAGPIX®, Luminex® 100/200™, and FLEXMAP 3D® instruments. Designed for ease-of-use, the software allows either a novice or advanced user to set up and run an assay in a minimal amount of time. All Luminex instrumentation sold by Bio-Techne arrives pre-installed with xPONENT data acquisition and analysis software.

The software's graphical user interface follows the typical assay workflow, but can be customized to meet the needs of a specific laboratory. Navigation wizards and automated routine operations such as startup, shutdown, calibration, and performance verification further enhance system usability and result in increased walk-away time.

xPONENT has many useful features that allow the user to generate high-quality data. Follow the steps outlined below to ensure the data is of the highest quality. Because each user's data are unique, analysis methods should be established based on the needs of each user.

Examine the Raw Data

1. Before and during acquisition.

   Take care to avoid errors in the assay* or instrument setup. These can include:

   • Pipetting errors

   • Washing errors or washer malfunctions

   • Improperly adjusted probe height

   *Refer to the assay kit product insert for the protocol and settings and make any necessary adjustments.

   Look for the following indicators of problems during the acquisition stage:

   • Slow bead acquisition

   • Timeout or "sample empty" warnings

   • Abnormal appearing scatter-plots

   • Shifts in the doublet discriminator or additional peaks

2. Post-acquisition.

   • Ensure that bead counts are at least 50 per analyte

Examine the Standard Curve

1. Visually inspect the standard curve (a 4-PL or 5-PL fit is recommended):

    -   The curve is best viewed in log x and log y scales

    -   Look for flattening of the curves at the top and bottom of the range

    -   Look for duplicate standard point separation (if plotted separately) or high %CVs

    -   Look for abnormal curve fits such as points off the line

    -   Note the R^2 values for the quality of fit

Based on these observations, use the following guidelines to identify any spurious points or data anomalies and edit if necessary.

Validate the Standard Curve

1. An acceptable standard curve will generally have these characteristics:

    -   A relatively linear shape that may be slightly curvilinear

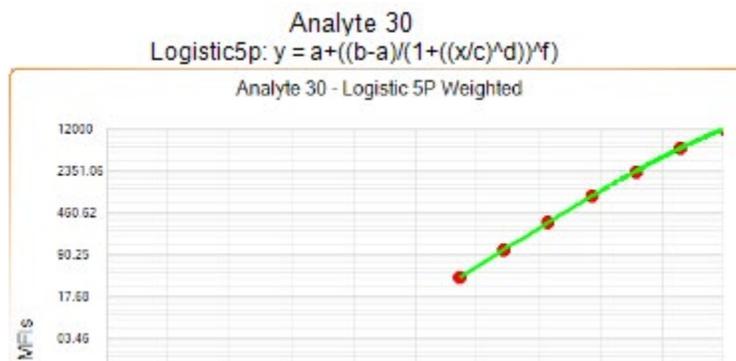   -   All points are touched by the line; see the example shown in figure 1

Figure 1. Linear Shape; All Points Touched by the Standard Curve Line

2. Look for separation between the standard points at the top and bottom of the curves. Significant loss of separation (flattening) can effect reproducibility and accuracy of the results. See the example shown in figure 2.
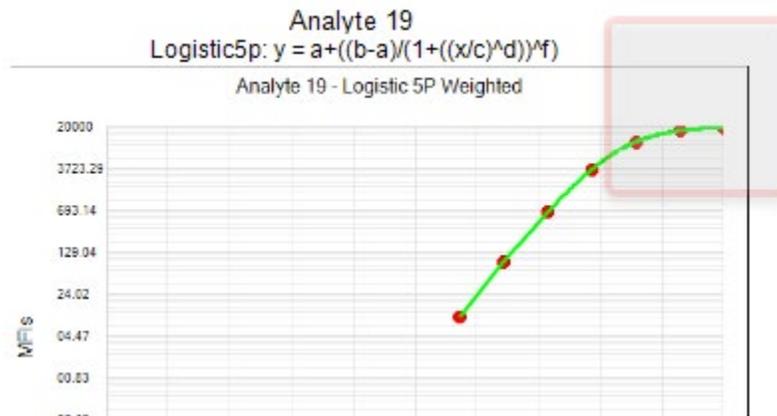

Figure 2. . Significant Loss of Separation (Flattening) between Points

2. a. If flattening is apparent at the top end of the standard curve, determine the severity and the need for editing.

   Guideline: If the difference between the top two standard points (S1 and S2) is <10%** of the Net MFI of the highest point (S1), consider removing or invalidating the S1 point.

   Example:
   Given: The S1 Net MFI = 11,000; S2 Net MFI = 10,000.

   10% of S1 Net MFI: 11,000 x 0.1= 1,100.

   Difference S1 Net MFI-S2 Net MFI: 11,000 - 10,000 = 1,000.

   Conclusion: 1,000 < 1,100 - Remove or invalidate the S1 point and re-analyze.

   b. If flattening is apparent at the bottom end of the standard curve, determine the severity and the need for editing.

   Guideline: The Net MFI signal of the low standard points (S6 or S7) should be $\geq$ 10 MFI**.

   Example:
   Given: Low standard curve flattening is apparent; Std 7 Net MFI = 4 MFI.

   Conclusion: Remove or invalidate the S7 point and re-analyze.

3. Examine the standard curve points for poor replication or outliers. This can be found in the Data Interpretation Report tables in xPONENT and are reported as %CVs of replicates [%CV = (STDev/Mean) × 100]. See the example shown in figure 3.

Net MFI	Test Result	Range	% Recovery	%CV of Replicates
21086	4797.338		95.947	
21673.5	5565.799		111.316	
13123.5	1622.031		97.322	
13316	1654.265		99.256	
4092	564.977		101.696	
3997.5	555.797		100.044	
691	186.679		100.807	
688	186.208		100.552	
97	61.276		99.268	
93	59.844		96.947	
15	21.272		103.381	
14	20.437		99.324	
3	7.811		113.884	
2	5.748		83.811	
21379.75	5181.569			10.487
13219.75	1638.148			1.391
4044.75	560.387			1.158
689.5	186.444			0.178
95	60.56			1.673
14.5	20.855			2.83
2.5	6.78			21.513

Figure 3. Data Interpretation Report (%CV of Replicates)

   Guideline: %CVs > 10%** may require editing of one of the standard point replicates.

   Example:
   Given: The low standard 7 Net MFI = 2.5 MFI; %CV is 21.5%.

   Conclusion: Remove or invalidate one of the S7 replicates and re-analyze.

4. % Recovery is one way to examine the quality of the curve fit. This data can be found in the Data Interpretation Report tables in xPONENT and are reported as % Recovery [% Recovery = (Observed Standard Value/Expected Standard Value) × 100]. In simple terms, this describes how well the standard points fit the chosen curve fitting model (4-PL or 5-PL) or if any points are off the line. See the example shown in figure 4.

Expected Conc	Net MFI	Test Result	Range	% Recovery	%CV of Replicates	Unit	
5,000.00	14571	4927.209		98.544		pg/mL	
1,666.67	9956	1661.054		99.663		pg/mL	
555.56	4452	560.429		100.877		pg/mL	
185.19	1334	183.682		99.188		pg/mL	
61.73	360	63.629		103.08		pg/mL	
20.58	86	20.799		101.084		pg/mL	
6.86	22	7.123		103.85		pg/mL	
5,000.00	14582	4945.638		98.913		pg/mL	E

Figure 4. Data Interpretation Report (% Recovery)

Guideline: % recovery of the standard point is in the range of 80–120%**.

Example:
Given: % recovery is within guidelines for all standard points

Conclusion: The curve model is appropriate; no editing is needed.

5. Finally, a review of the curve fitting parameters and most importantly the R^2 statistic is a critical step. This may be done early in the curve review process, or as each editing step is taken to assess improvement. R^2 is a commonly used statistic designed to give an overall assessment of the goodness of fit of the data using the chosen model. This can also be found on the Data Interpretation Report in xPONENT. See the example shown in figure 5.
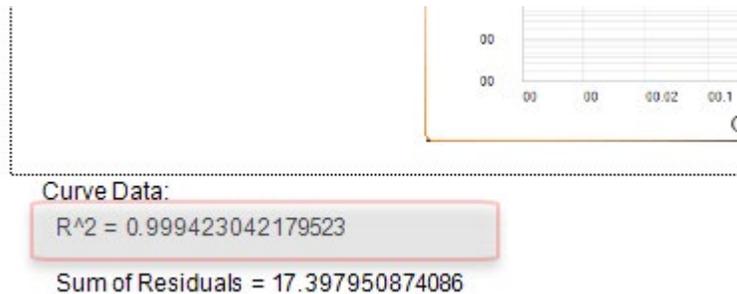

Curve Data:
R^2 = 0.999423042179523

Sum of Residuals = 17.397950874086

Figure 5. Data Interpretation Report (R2)

Guideline: : $R^2 > 0.95$**

Example:
Given: Data yields an R^2 of 0.999.

Conclusion: The curve model is appropriate; no editing is needed.

Examine the Sample Data

Though some of the guidelines defined above can apply to the sample/unknown data, there are fewer parameters on which to base decisions regarding the quality of the data. For that reason, it is recommended that those decisions be limited to review of replicates (%CVs). As always, acceptance criteria must be established by each user.

Notes on Invalidating or Editing the Data

Data can be edited and re-analyzed by one of two means:

1. If using a secure version of the xPONENT software, the individual data points can be invalidated from the results tab. Administrator privilege is required to use this feature.

2. Data can be edited by applying a modified protocol and choosing a replay of the batch data.

Refer to the xPONENT user guide for details.

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