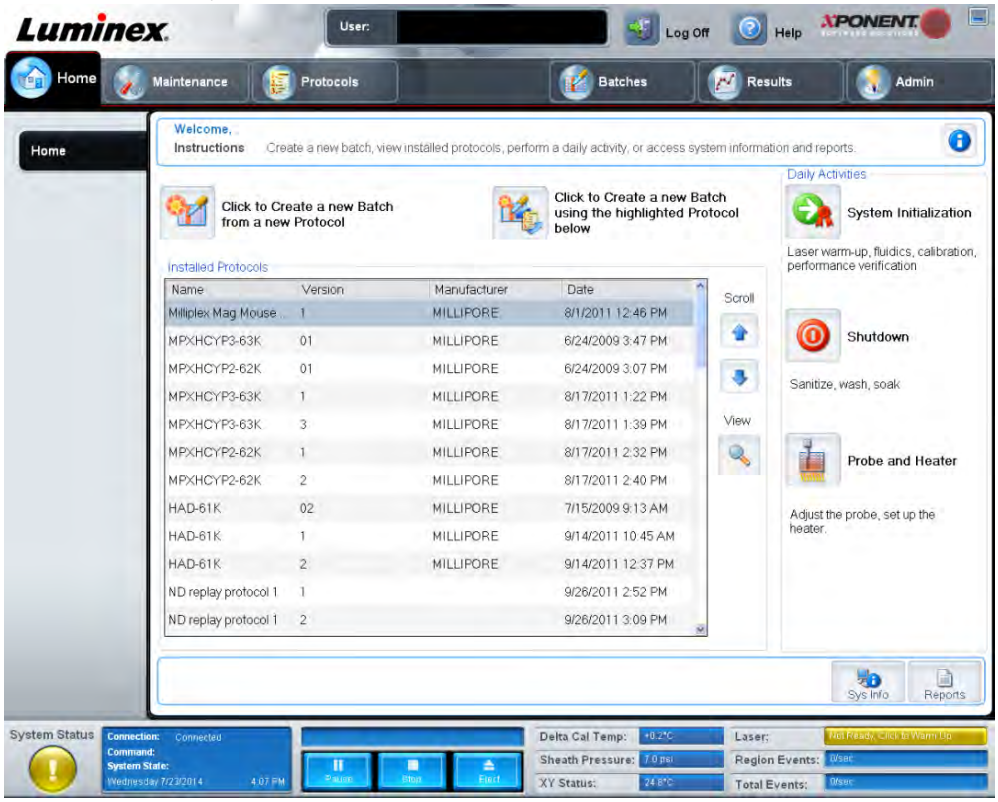
Luminex 200 xPONENT 3.1 Instrument Setup Protocol

Introduction: This protocol is a step-by-step method for setting up the xPONENT 3.1 software for running Luminex assays.

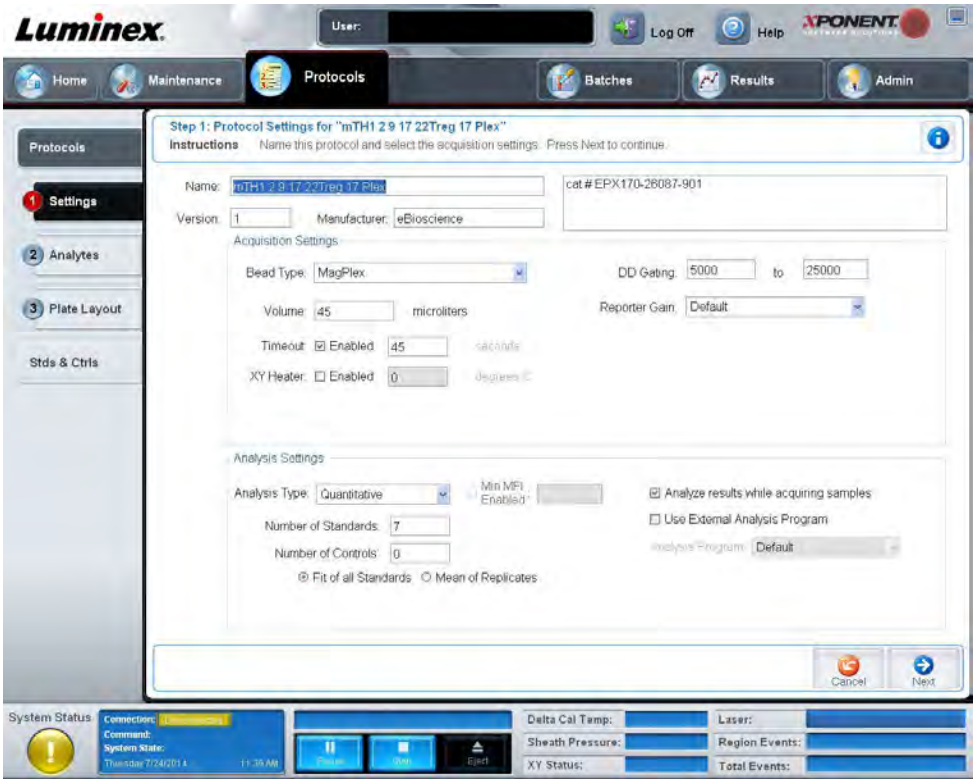
Luminex technology employs microsphere beads, either polystyrene or paramagnetic, that are color-coded. 500 distinct bead color sets are available. Each bead set can be coated with a reagent specific to a particular bioassay, permitting the capture and detection of specific analytes from a sample. Inside the Luminex analyzer, the classification laser (red, 635 nm) excites the internal dyes identifying each microsphere bead, and the reporter laser (green, 532 nm) excites any reporter dye (Phycoerythrin) captured during the assay. Luminex kits include standards for each analyte in the assay; a standard curve is generated for each analyte. Each sample value is extrapolated from a standard curve. Luminex kits can quantify nucleic acids and proteins. The KUMC Luminex 200 reads a 96 well plate in approximately 40 minutes, with a dynamic range of 3.5 logs.

Protocol (note: all Luminex commands and menu items indicated are in **bold typeface**):

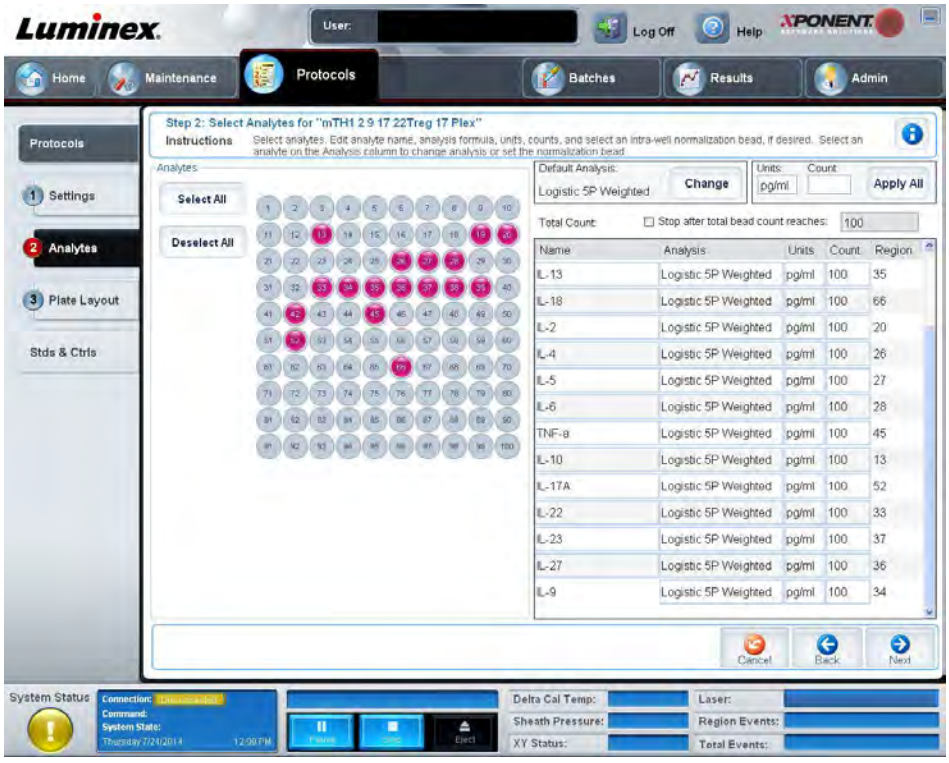
1. Start the Luminex xPONENT program from the Start menu or the desktop shortcut.
2. At the **System Login** screen, just click **Log In**. There is no **User ID**.
3. At the **Home** screen, click on the **Protocols** tab.



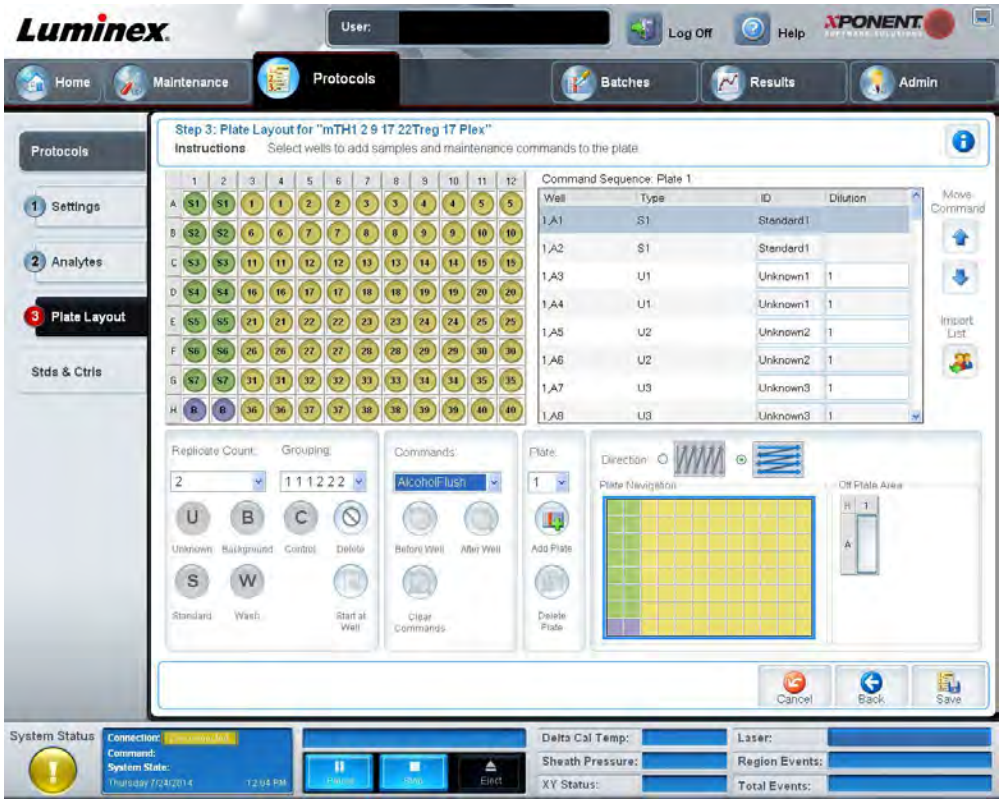
4. At the **Protocols** tab, click **Create New Protocol** (**Step 1**). On this screen, Luminex kit details are necessary. Enter the **Name** for the protocol, set a **Version** number. The kit manufacturer will provide the following details: **Volume** = Sample Size, **DD Gate**, and **Timeout**. The **DD Gate** is the doublet discrimination window. Fill in the **Analysis Settings**. Finally, click **Next**.



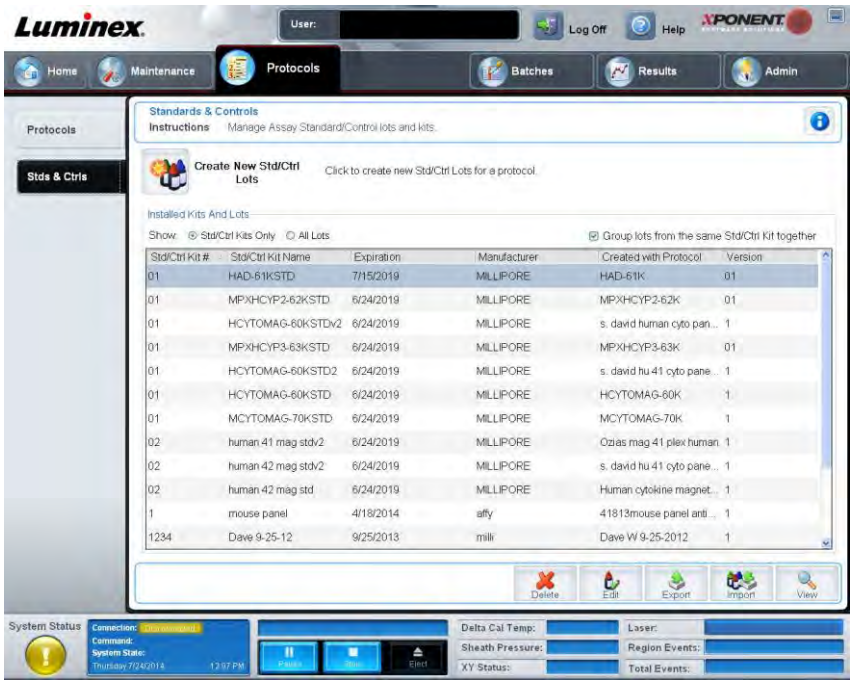
5. The Luminex kit will come from the manufacturer with a specific number assigned to each analyte (**Step 2**). Select each number and fill in the analyte name in the **Name** field. For instance, in this kit, IL-13 is analyte number 35. Set **Units** to the proper value (usually, pg/ml). Click **Apply All**, then **Next**. The default analysis is a 5 parameter fit.



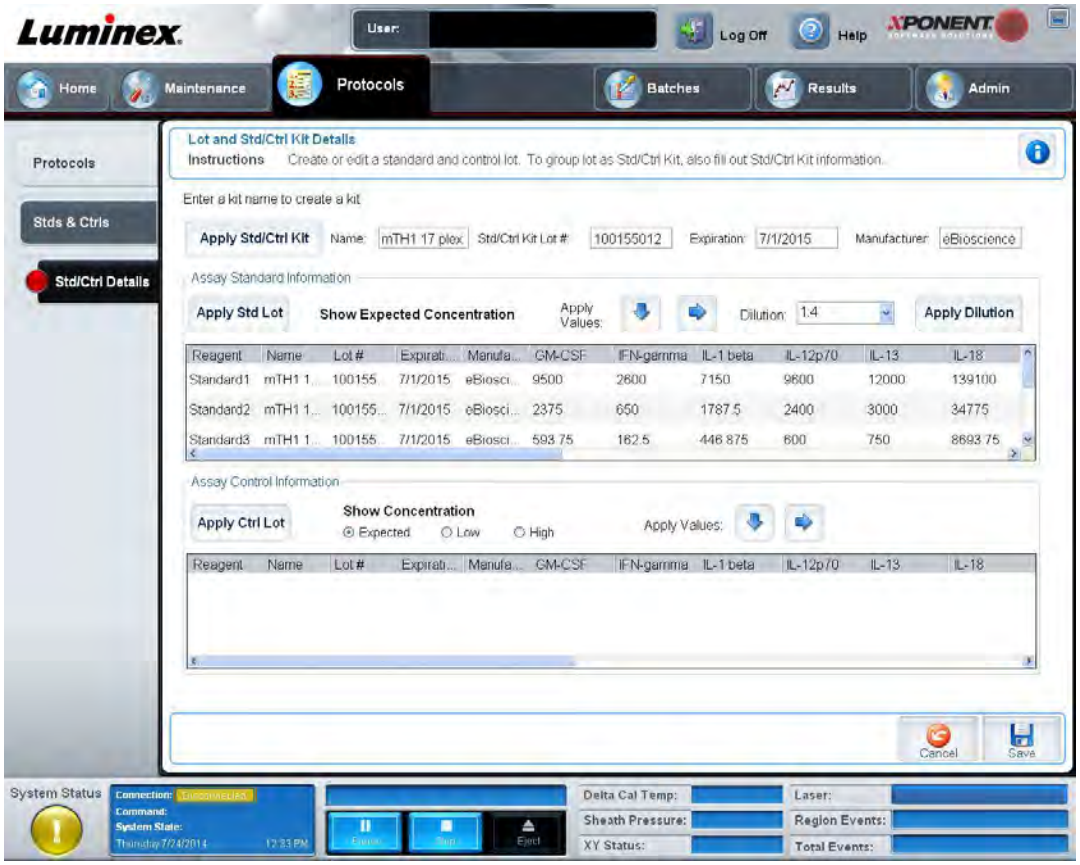
6. The **Plate Layout** screen is for setting up the plate (**Step 3**). Define the **Standards, Blanks, Unknowns, Replicate Count, Dilution** and plate orientation. Click **Save**.



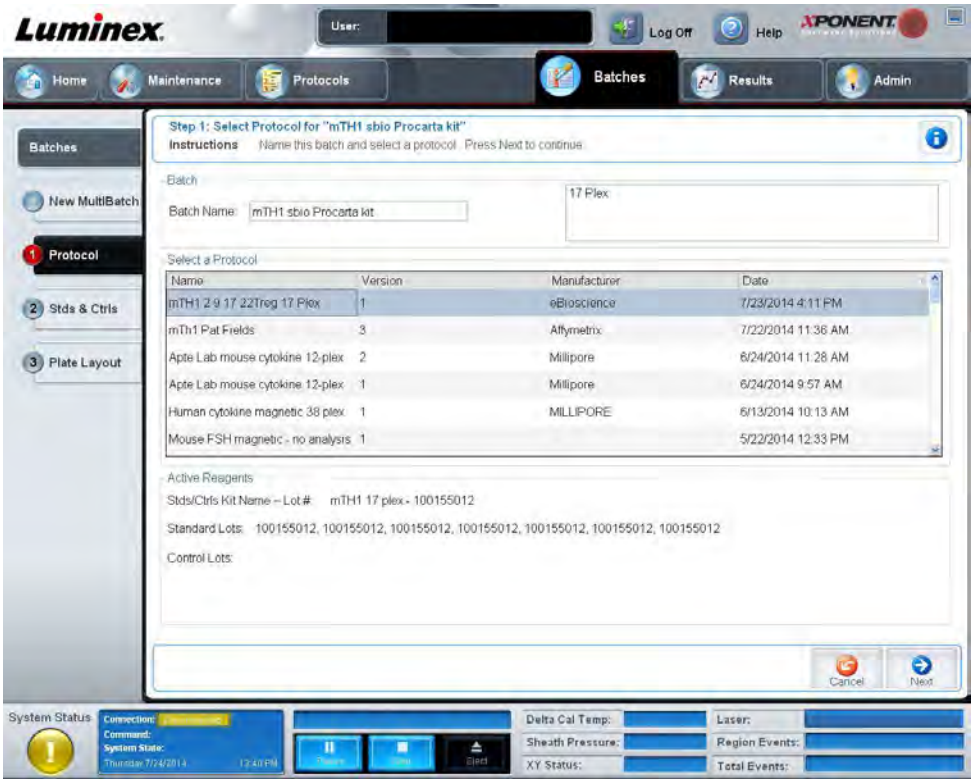
7. Click on the **Stds & Ctrls** tab. Click Create **New Std/Ctrl Lots**.



8. The program will ask for the name of the Protocol; select the appropriate protocol and click **OK**. Create a **Name** for the standards; enter the **Std/Ctrl Kit Lot #**, **Expiration** date and the **Manufacturer** information. Go to the **Assay Standard Information** menu; in the first row under the name of each analyte enter the concentration provided by the manufacturer. In the **Dilution** box, type the whole number of your dilution scheme. For example, here we typed 4, selected all analytes, then clicked **Apply Dilution** and the program listed the Dilution as 1:4. Click **Save**.



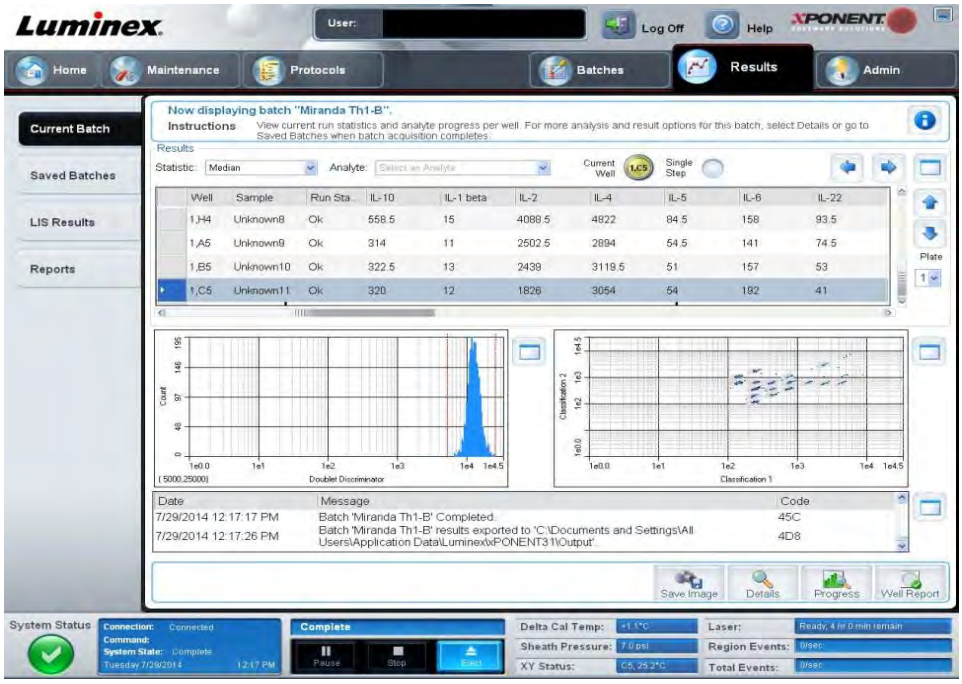
9. Go to the **Batches** tab. Click **Create a New Batch** in **Step 1**. Create a **Batch Name**. Enter a **Description.** Under **Select a Protocol**, find your protocol and then click **Next.**



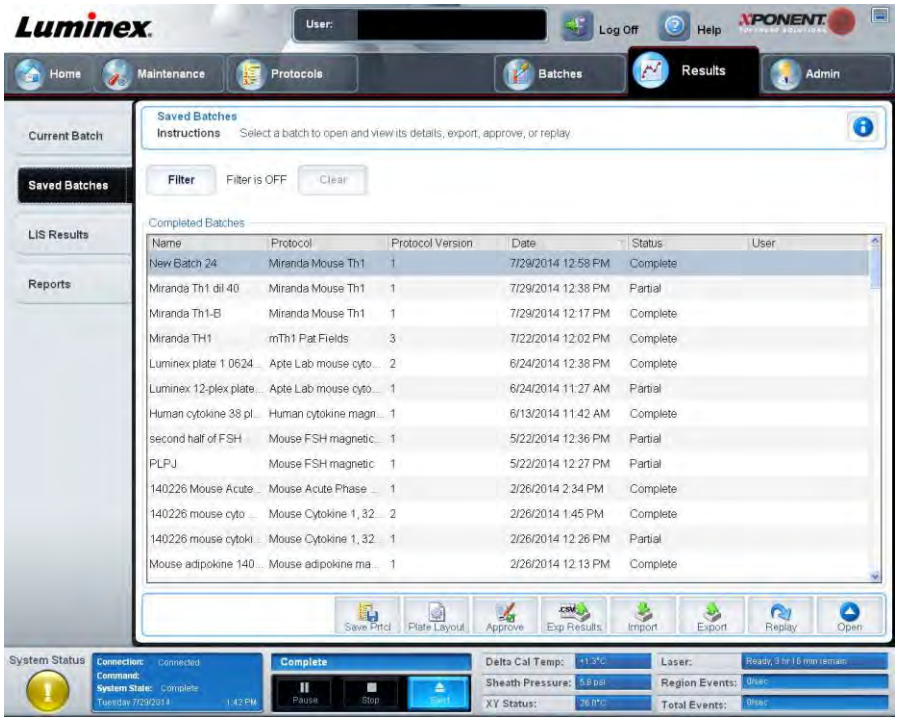
10. **Step 2** displays the **Stds & Ctrls** associated with this Batch and Protocol. Then click **Next.**

11. **Step 3** displays the **Plate Layout**. Verify the plate layout; insert the plate into the instrument. Click **Run Batch**.

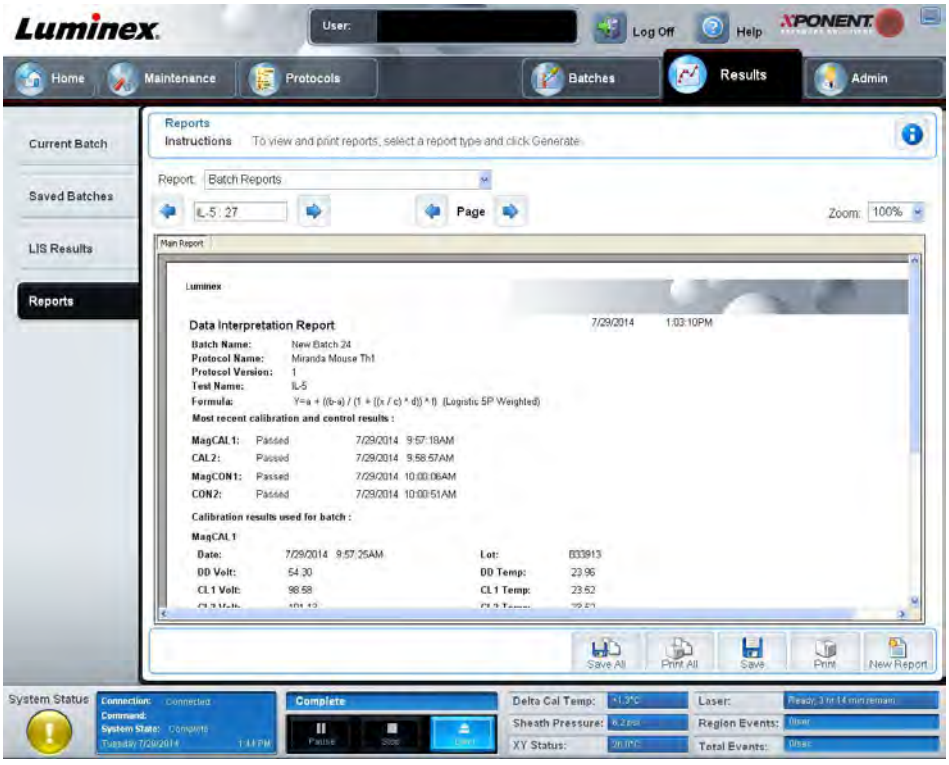
12. The plate is now running. The **Doublet Discriminator** histogram displays the single beads as they pass through the classification laser, doublets are excluded. The **Classification 1** versus **Classification 2** dot plot displays the dyed beads for each analyte (in this case, 17 different analytes.)



13. After the batch plate runs; click the **Saved Batches** tab. Navigate to and highlight the correct **Batch Name** and click **Exp Results**.



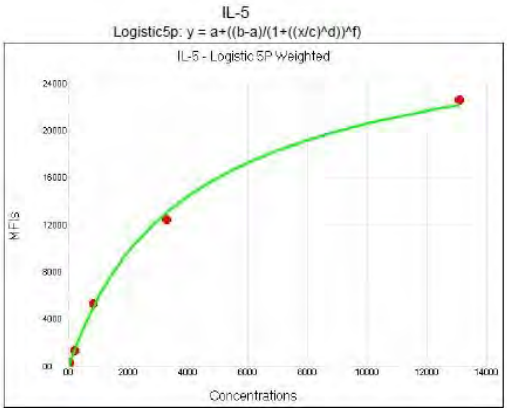
14. Next click the **Reports** tab; select **Save All**. Reports will be generated for each analyte. The reports will contain the computed values for each unknown sample and a standard curve for each analyte.



15. Report output: standard curve, and tables.

Data Interpretation Report 7/29/2014

Batch Name:	New Batch 24
Protocol Name:	Miranda Mouse Th1
Protocol Version:	1
Test Name:	IL-5



Standards:

Loc	Standard	Expected Conc	Net MFI	Test Result	Range	% Recovery	%CV of Replicates	Unit	Comments
1,A3	Standard1	13,100.00	22557	13955.01		106.527		pg/ml	
1,B3	Standard2	3,275.00	12014.5	3042.784		92.909		pg/ml	
1,D3	Standard4	204.69	1214.5	209.129		102.17		pg/ml	
1,C3	Standard3	818.75	4828	853.319		104.222		pg/ml	
1,G3	Standard7	3.20	13	3.169		99.095		pg/ml	
1,F3	Standard6	12.79	50	13.215		103.3		pg/ml	
1,E3	Standard5	51.17	230	48.945		95.648		pg/ml	

Samples:

Loc	Sample ID	Net MFI	Test Result	Range	%CV of Replicates	Unit	Dilution Factor	Comments
1,H3	Background0	0					0.00	
1,A4	Unknown1	69	702.564			pg/ml	40.00	
1,C4	Unknown2	29.5	322.967			pg/ml	40.00	
1,B4	Unknown3	25.5	279.222			pg/ml	40.00	
1,D4	Unknown4	51	538.075			pg/ml	40.00	
1,G4	Unknown7	25	273.632			pg/ml	40.00	
1,F4	Unknown6	38	411.334			pg/ml	40.00	
1,E4	Unknown5	48	509.541			pg/ml	40.00	
1,A5	Unknown9	37	401.206			pg/ml	40.00	
1,H4	Unknown8	62	639.807			pg/ml	40.00	
1,C5	Unknown11	31	338.972			pg/ml	40.00	
1,B5	Unknown10	33	360.017			pg/ml	40.00	

16. The .csv file can also be found in the xPonent output folder (located on the desktop). KUMC flow core staff will run your plate through the Millipore Analyst Luminex data analysis program. Analyst does not automatically bring in the xPonent dilutions, we must set them in the Analyst plate page.