

Luminex® 200 (w/ xPONENT 4.3) Setup and Data Acquisition for RayPlex® Arrays

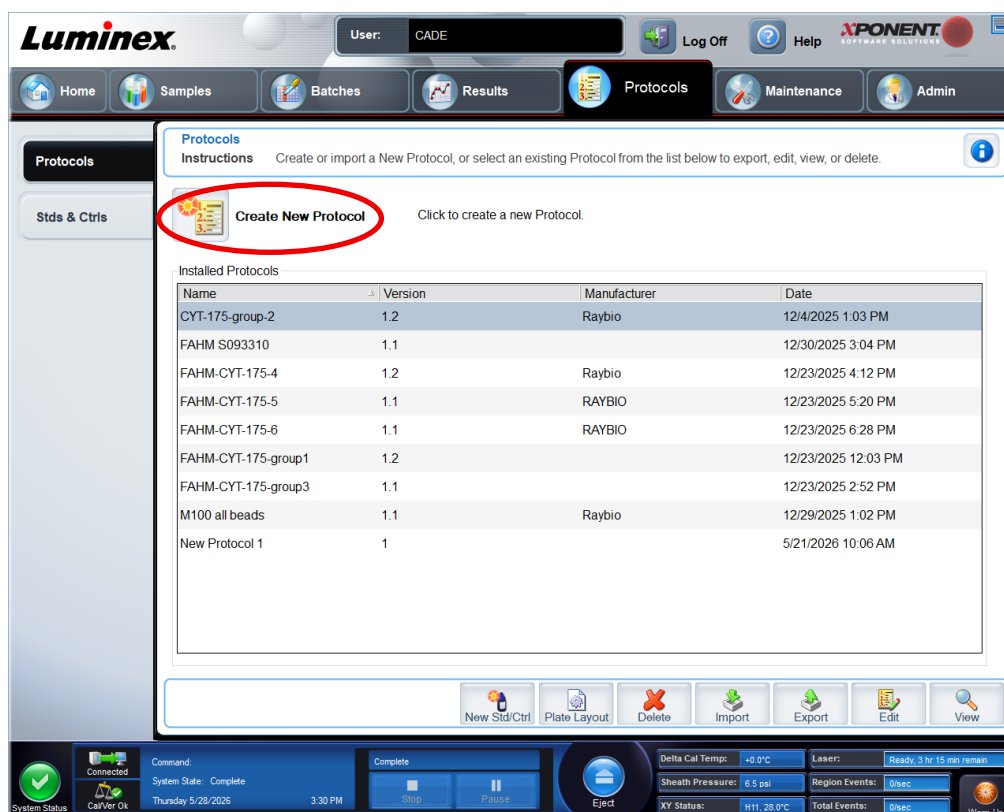
Note: The bead set included with Luminex-compatible RayPlex assays has been tested by RayBiotech and shown to function on compatible Luminex/xMAP instruments under the conditions described in this manual. The bead set is not manufactured, licensed, approved, endorsed, or authorized by Luminex. Because these assays use non-Luminex-approved beads, use of this product may void or limit the instrument warranty, service contract, or service support, depending on the terms established by Luminex or the user's service provider. Users should review their applicable instrument warranty and service agreement before use.

Note: These instructions are general for all Luminex-compatible RayPlex assays. The target count, bead size, and populations may differ. Please see the Bead ID and Distribution section in the kit manual for the bead size and populations present in your array.

Perform standard QC and optimization steps to prepare the Luminex instrument and then set up the instrument as you would for normal use.

Part 1: Creating a New Protocol

1. From the **Home** screen, navigate to the **Protocols** tab.



2. Click **Create New Protocol (Step 1)**. In the **Settings** panel, configure the following parameters — the RayPlex kit documentation will supply kit-specific values:
 - **Protocol Name:** Enter a descriptive name.

- **Sample Volume:** 50 μ L (recommended)
- **DD Gate:** 5,000–17,000 (RayPlex default; this is the doublet discrimination window used to gate single bead events)
- **Timeout:** 60 seconds
- **Analysis Type:** Quantitative
 - Number of Standards: 7
 - Number of Controls: 0
- **Version:** Enter a version number for tracking purposes.

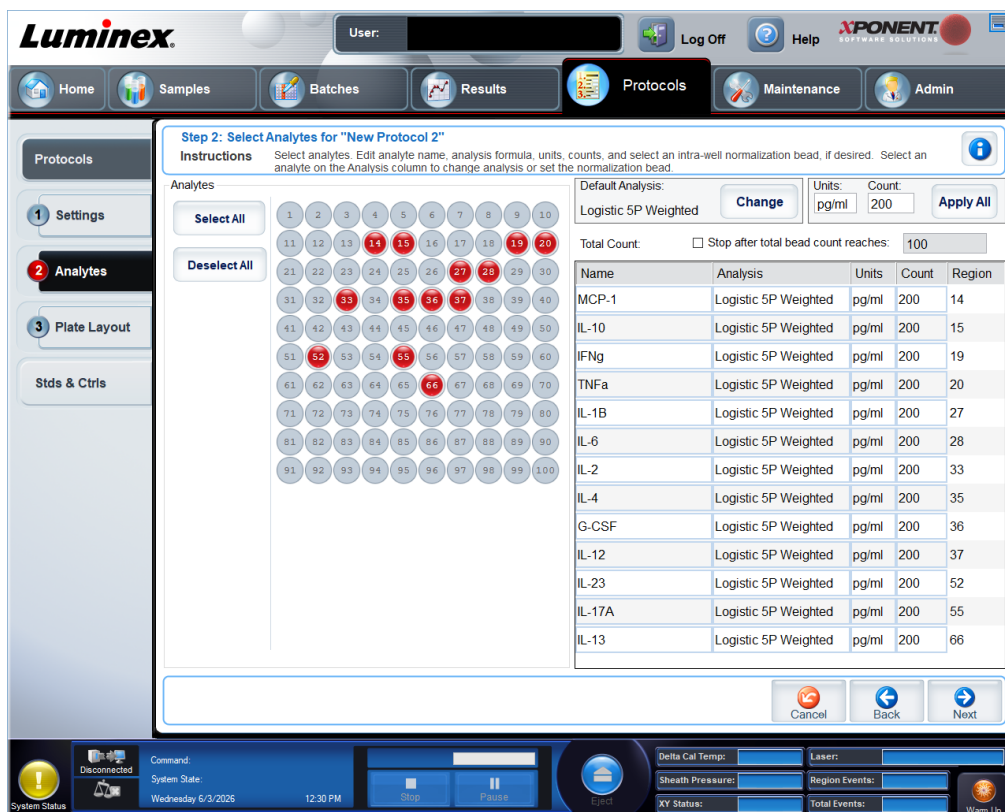
Click **Next** when finished.

3. On the **Analytes** tab, assign each Bead ID to its corresponding analyte. Bead region numbers are derived from the kit documentation — each analyte is assigned an "M-number" (e.g., M43 = bead region 43).

For each analyte listed in the kit:

- Locate and select the corresponding bead region number
- Enter the analyte name in the **Name** field
- **Curve Fit:** 5-Parameter Logistic (5PL) — default
- **Units:** pg/mL (default; adjust if needed)
- **Bead Count:** Set the minimum bead count per region per the kit's recommendation.

Click **Apply All**, then **Next**.



- On the **Plate Layout** tab, define the plate configuration. Assign wells to Standards, Blanks, and Unknowns. Set the **Replicate Count**, **Dilution** and plate **Direction**. Click **Save**.



- Navigate to the **Stds & Ctrl** tab and click **Create New Std/Ctrl Lots**. When prompted, select the appropriate protocol and click **OK**.
 - Enter a name for the standard lot

- In the **Std/Ctrl Detail** window, fill in the Kit Name, Lot Number, Expiration Date and Manufacturer (e.g. RayBiotech).
- Under **Assay Standard Information**, enter the same kit details in the header row, then use **Apply Values** to propagate this information to all remaining rows.
- In the first data row, enter the top-standard concentration for each analyte as provided in the kit documentation.
- In the **Dilution** field, enter the serial dilution factor (e.g. 1:2, 1:3, or 1:5), then click **Apply Dilution** to auto-populate the remaining standard concentrations.

Note: If a Std/Ctrl lot has already been configured in a saved protocol, you only need to set the batch name and click **Apply Std/Ctrl Kit** to link the existing standards.

Click **Save**.

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Home Samples Batches Results Protocols Maintenance Admin

Standards & Controls
Instructions Manage Assay Standard/Control lots and kits.

Std/Ctrl **Create New Std/Ctrl Lots** Click to create new Std/Ctrl Lots for a protocol.

Installed Kits And Lots

Show: ☒ Std/Ctrl Kits Only ☐ All Lots ☒ Group lots from the same Std/Ctrl Kit together

Std/Ctrl Kit #	Std/Ctrl Kit Name	Expiration	Manufacturer	Created with Protocol	Version
120425	FAHM-CYT-group2	12/4/2027	Raybio	CYT-175-group-2	1.1
120425	New protocol05212026	12/4/2027	Raybio	New Protocol 1	1
2025	FAHM-CYT-175-3	12/23/2027	Raybio	FAHM-CYT-175-group3	1.1
2025	FAHM-CYT-175-group1	12/23/2026	RAYBIO	FAHM-CYT-175-group1	1.2
2025 DEC	FAHM-CYT-175-4	12/23/2027	Raybio	FAHM-CYT-175-4	1.2
20251223	FAHM-CYT-175-6	12/23/2027	RAYBIO	FAHM-CYT-175-6	1.1
20251223	FAHM-CYT-175-5	12/23/2027	RAYBIO	FAHM-CYT-175-5	1.1

Delete Edit Export Import View

System Status: Connected Command: Complete System State: Complete Thursday 5/28/2026 3:44 PM Complete Stop Pause Eject

Delta Cal Temp: -0.0°C Laser: Ready, 3 hr 1 min remain
Sheath Pressure: 6.5 psi Region Events: 0/sec
XY Status: 28.2°C Total Events: 0/sec Warm Up

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Protocols

Std/Ctrl Kit Details

Lot and Std/Ctrl Kit Details
Instructions Create or edit a standard and control lot. To group lot as Std/Ctrl Kit, also fill out Std/Ctrl Kit information.

Enter a kit name to create a kit

Apply Std/Ctrl Kit Name: Std/Ctrl Kit Lot #: Expiration: Manufacturer:

Assay Standard Information

Apply Std Lot Show Expected Concentration Apply Values: Dilution: 1:2 **Apply Dilution**

Reagent	Name	Lot #	Expirati...	Manufa...	Analyte 4	Analyte 8	Analyte 18	Analyte 30	Analyte 33	Analyte 34
Standard1										
Standard2										
Standard3										

Assay Control Information

Apply Ctrl Lot Show Concentration ☒ Expected ☐ Low ☐ High Apply Values:

Reagent	Name	Lot #	Expirati...	Manufa...	Analyte 4	Analyte 8	Analyte 18	Analyte 30	Analyte 33	Analyte 34

Cancel Save

System Status Connected Command: Complete Thursday 5/28/2025 3:49 PM Delta Cal Temp: -0.2°C Laser: Ready, 2 hr 56 min remain Sheath Pressure: 6.5 psi Region Events: 0/sec XY Status: 28.2°C Total Events: 0/sec Warm Up

Part 2: Creating and Running a Batch

6. Go to the **Batches** tab and click **Create a New Batch** (Step 1).

- Enter a **Batch Name** and optional **Description**.
- Under **Select a Protocol**, locate and select your protocol.

Click **Next**.

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Batches

Batches
Instructions Create new batches with or without a protocol, or manage and run a pending batch.

Create a New Batch from an existing Protocol Click to create a new batch from an existing protocol.

Create a New Batch from a new Protocol Click to create a new batch without using a protocol.

Create a New Multi-Batch Click to create a new multi-batch from pending batches.

Pending Batches

Name	Protocol	Protocol Version	Date	Status
New Batch 4	FAHM-CYT-175-4	1.1	12/23/2025 4:11 PM	Partial
New Batch 1	FAHM-CYT-175-group1	1.1	12/23/2025 12:00 PM	Partial

Single Step

Reacquire Save Prcf Plate Layout Import Export Delete Edit Run

System Status Connected Command: Complete Thursday 5/28/2025 3:49 PM Delta Cal Temp: -0.2°C Laser: Ready, 2 hr 55 min remain Sheath Pressure: 6.5 psi Region Events: 0/sec XY Status: 28.3°C Total Events: 0/sec Warm Up

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Home Samples **Batches** Results Protocols Maintenance Admin

Batches

1 Protocol

2 Stds & Ctrls

3 Plate Layout

Step 1: Select Protocol for "New Batch 6"

Instructions Name this batch and select a protocol. Press Next to continue.

Batch

Batch Name: New Batch 6 *Enter optional description here*

Select a Protocol

Name	Version	Manufacturer	Date
FAHM-CYT-175-6	1.1	RAYBIO	12/23/2025 6:28 PM
FAHM-CYT-175-group1	1.2		12/23/2025 12:03 PM
FAHM-CYT-175-group3	1.1		12/23/2025 2:52 PM
M100 all beads	1.1	Raybio	12/29/2025 1:02 PM
M100 All Beads	1		5/28/2026 3:44 PM
New Protocol 1	1		5/21/2026 10:06 AM

Active Reagents

Sids/Ctrls Kit Name – Lot #: m100 all beads - 05282026

Standard Lots: 05282026, 05282026, 05282026, 05282026, 05282026, 05282026, 05282026

Control Lots:

Cancel Next

System Status Connected Command: Complete System State: Complete Thursday 5/28/2025 3:50 PM Stop Pause Eject

Delta Cal Temp: +0.0°C Laser: Ready, 2 hr 55 min remain Sheath Pressure: 6.5 psi Region Events: 0/sec XY Status: 28.3°C Total Events: 0/sec Warm Up

7. **Step 2** displays the Standards and Controls associated with the selected protocol. Verify the information is correct, then click **Next**.

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Home Samples **Batches** Results Protocols Maintenance Admin

Batches

1 Protocol

2 Stds & Ctrls

3 Plate Layout

Step 2: Set up Reagents for "M100 Run"

Instructions Select lots for this batch.

Apply Std/Ctrl Kit Name: m100 all be... Std/Ctrl Kit Lot #: 05282026 Expiration: 5/28/2031 Manufacturer: RayBio

Assay Standard Information

Apply Std Lot Show Expected Concentration Apply Values: Dilution: 1:2 Apply Dilution

Reagent	Name	Lot #	Expirati...	Manufa...	Analyte 4	Analyte 8	Analyte 9	Analyte 14	Analyte 15	Analyte 18
Standard1		05282026	5/28/2031	RayBio	200	200	200	200	200	200
Standard2		05282026	5/28/2031	RayBio	66.666666...	66.666666...	66.666666...	66.666666...	66.666666...	66.666666...
Standard3		05282026	5/28/2031	RayBio	22.222222...	22.222222...	22.222222...	22.222222...	22.222222...	22.222222...
Standard4		05282026	5/28/2031	RayBio	7.4074074...	7.4074074...	7.4074074...	7.4074074...	7.4074074...	7.4074074...

Assay Control Information

Apply Ctrl Lot Show Concentration Expected Low High Apply Values:

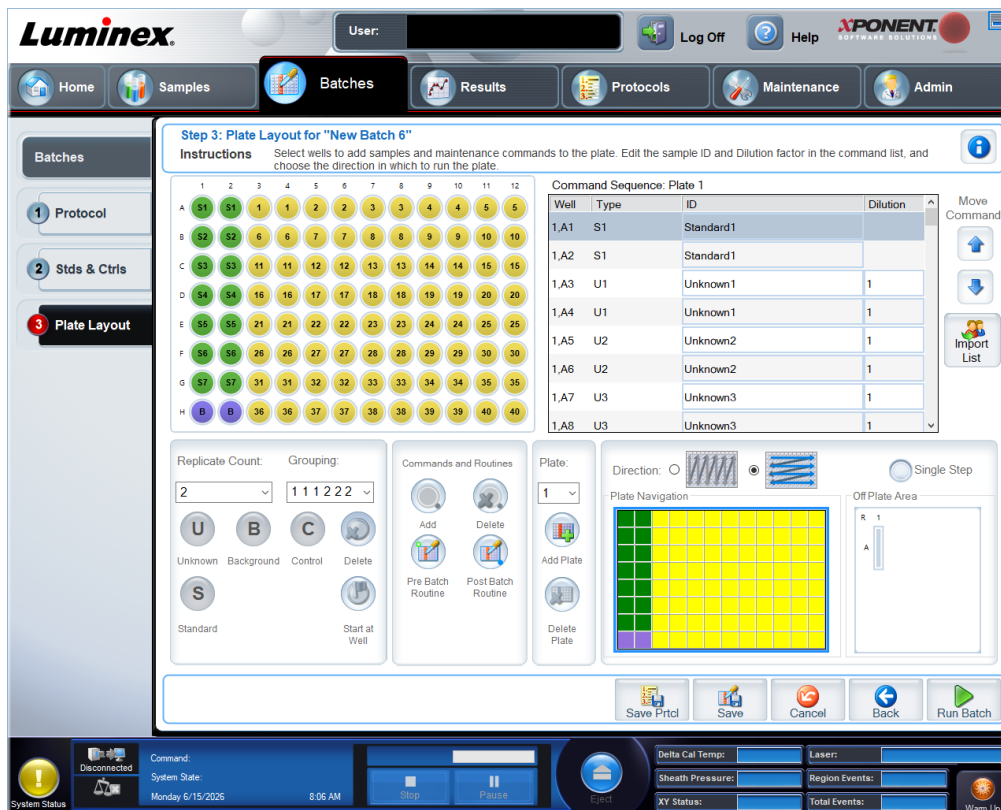
Reagent	Name	Lot #	Expirati...	Manufa...	Analyte 4	Analyte 8	Analyte 9	Analyte 14	Analyte 15	Analyte 18

Cancel Back Next

System Status Connected Command: Complete System State: Complete Thursday 5/28/2025 3:51 PM Stop Pause Eject

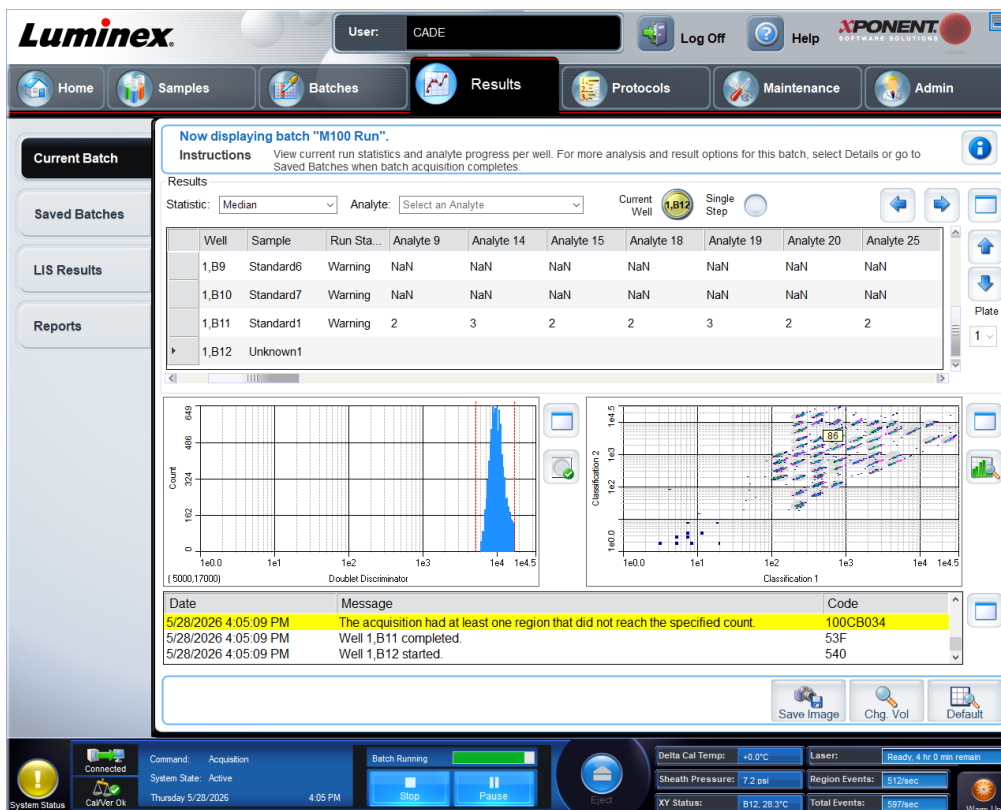
Delta Cal Temp: +0.0°C Laser: Ready, 2 hr 54 min remain Sheath Pressure: 6.5 psi Region Events: 0/sec XY Status: 28.3°C Total Events: 0/sec Warm Up

8. **Step 3** displays the **Plate Layout**. Confirm the layout matches your plate setup, then load the plate into the instrument. Click **Run Batch** to begin acquisition.



9. During the run, monitor the following displays:

- **Doublet Discriminator (DD) histogram** – shows singlet bead events as they pass through the classification laser; doublet events are excluded based on the DD gate set in Step 2.
- **Classification 1 vs. Classification 2 dot plot** – displays the spectrally distinct bead populations for each analyte (e.g., 17 analyte regions for a 17-plex panel).



Part 3: Reviewing Results and Exporting Data

10. After the run is complete, navigate to the **Saved Batches** tab. Highlight the appropriate batch and click **Exp Results** to open the results view.

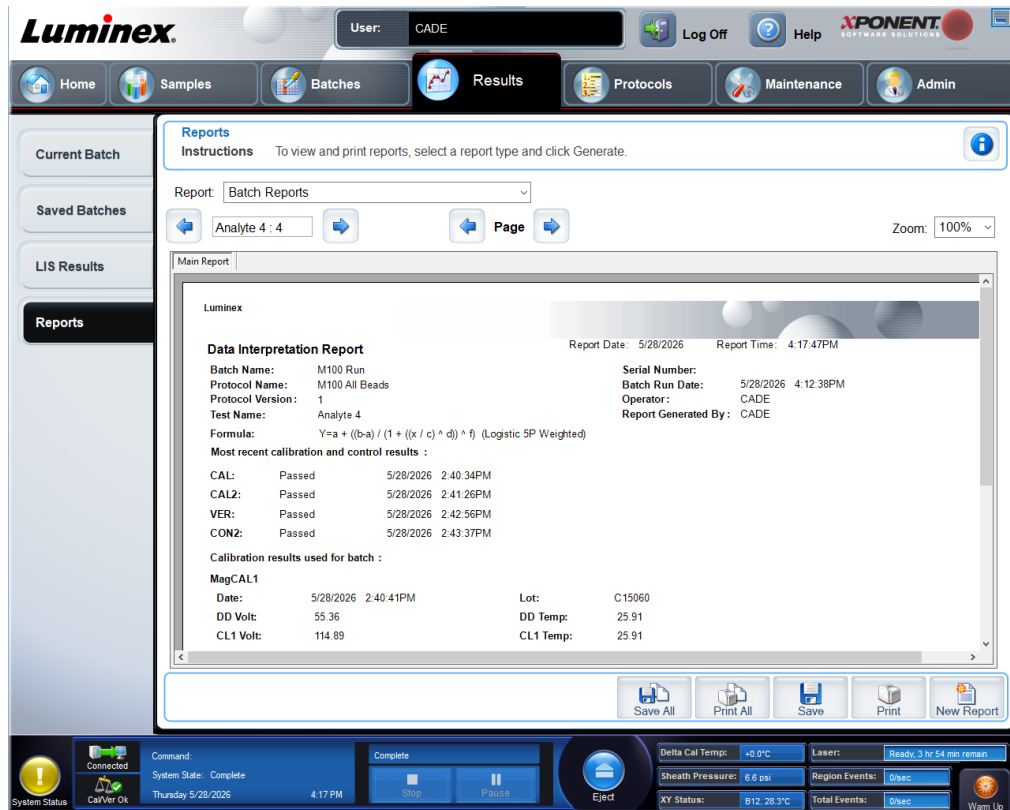
The screenshot shows the Luminex software interface with the **Saved Batches** tab selected. The interface includes a top navigation bar with tabs for Home, Samples, Batches, Results, Protocols, Maintenance, and Admin. The **Saved Batches** section on the left sidebar is active. The main area displays a table of completed batches with columns for Name, Protocol, Protocol Version, Date, Status, and User. Below the table are buttons for Reacquire, Save Pctl, Plate Layout, Approve, Exp Results, Import, Export, Replay, and Open. The bottom status bar shows system information and control buttons.

Name	Protocol	Protocol Version	Date	Status	User
M100 Run	M100 All Beads	1	5/28/2026 4:12 PM	Complete	CADE
M100 Run - Copy	M100 All Beads	1	5/28/2026 4:06 PM	Complete	CADE
FAHM S093310 P2	FAHM S093310	1.1	12/30/2025 3:41 PM	Complete	Cade
New Batch 5	FAHM S093310	1.1	12/30/2025 3:30 PM	Complete	Cade
12-29-25 M100 all be...	M100 all beads	1.1	12/29/2025 1:32 PM	Complete	Lewis
FAHM-CYT-175-6-20...	FAHM-CYT-175-6	1.1	12/23/2025 7:30 PM	Complete	
FAHM-CYT-175-5-20...	FAHM-CYT-175-5	1.1	12/23/2025 6:22 PM	Complete	
FAHM-CYT-175-4 12...	FAHM-CYT-175-4	1.2	12/23/2025 5:14 PM	Complete	
New Batch 4	FAHM-CYT-175-4	1.1	12/23/2025 4:11 PM	Partial	
New Batch 3	FAHM-CYT-175-group3	1.1	12/23/2025 3:55 PM	Complete	
FAHM-CYT-175-grou...	CYT-175-group-2	1.2	12/23/2025 2:41 PM	Complete	
New Batch 2	FAHM-CYT-175-group1	1.2	12/23/2025 1:21 PM	Complete	
New Batch 1	FAHM-CYT-175-group1	1.1	12/23/2025 12:00 PM	Partial	

11. Click the **Reports** tab and select **Generate**. A report will be generated for each analyte containing the computed concentration values for all unknown samples and a standard curve for the analyte.

The screenshot shows the Luminex software interface with the **Reports** tab selected. The **Reports** section on the left sidebar is active. The main area displays a table of completed batches, similar to the previous screenshot. To the right of the table is a **Select Analytes** list with checkboxes for various analytes. Below the table and list are buttons for **All**, **Clear**, and **Generate**. The bottom status bar shows system information and control buttons.

Name	Protocol	Protocol Version	Date	Status	User
M100 Run	M100 All Beads	1	5/28/2026 4:12 ...	Complete	CADE
M100 Run - Copy	M100 All Beads	1	5/28/2026 4:06 ...	Complete	CADE
FAHM S093310 ...	FAHM S093310	1.1	12/30/2025 3:41 ...	Complete	Cade
New Batch 5	FAHM S093310	1.1	12/30/2025 3:30 ...	Complete	Cade
12-29-25 M100 ...	M100 all beads	1.1	12/29/2025 1:32 ...	Complete	Lewis
FAHM-CYT-175-...	FAHM-CYT-175-6	1.1	12/23/2025 7:30 ...	Complete	
FAHM-CYT-175-...	FAHM-CYT-175-5	1.1	12/23/2025 6:22 ...	Complete	
FAHM-CYT-175-...	FAHM-CYT-175-4	1.2	12/23/2025 5:14 ...	Complete	
New Batch 4	FAHM-CYT-175-4	1.1	12/23/2025 4:11 ...	Partial	
New Batch 3	FAHM-CYT-175-...	1.1	12/23/2025 3:55 ...	Complete	
FAHM-CYT-175-...	CYT-175-group-2	1.2	12/23/2025 2:41 ...	Complete	
New Batch 2	FAHM-CYT-175-...	1.2	12/23/2025 1:21 ...	Complete	
New Batch 1	FAHM-CYT-175-...	1.1	12/23/2025 12:0 ...	Partial	
standard after ad...	CYT-175-group-2	1.2	12/4/2025 1:05 ...	Complete	ben



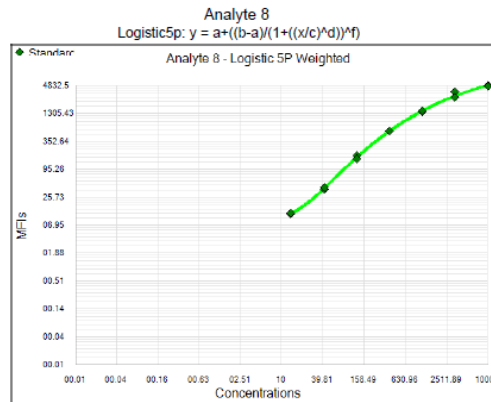
12. Report output:

Data Interpretation Report

Batch Name: FAHM-CYT-175-5-20251223
Protocol Name: FAHM-CYT-175-5
Protocol Version: 1.1
Test Name: Analyte 8

Report Date: 6/10/2026 Report Time: 9:01:22AM

Serial Number:
Batch Run Date: 12/23/2025 6:22:08PM
Operator:
Report Generated By:



Loc	Standard	Expected Conc	Net MFI	Test Result	Range	% Recovery	%CV of Replicates	Unit	Comments
1,A1	Standard1	10,000.00	4832.5	10422.83		104.228		pg/ml	Extrapolated
1,A2	Standard1	10,000.00	4568.5	8998.562		89.986		pg/ml	
1,B1	Standard2	3,333.33	3526.5	4938.997		148.17		pg/ml	
1,B2	Standard2	3,333.33	2707.5	2952.79		88.584		pg/ml	
1,C1	Standard3	1,111.11	1370.5	1038.147		93.433		pg/ml	
1,C2	Standard3	1,111.11	1430	1098.78		98.89		pg/ml	
1,D1	Standard4	370.37	562.5	372.78		100.651		pg/ml	
1,D2	Standard4	370.37	559.5	370.773		100.109		pg/ml	
1,E1	Standard5	123.46	179.5	133.756		108.343		pg/ml	
1,E2	Standard5	123.46	152.5	117.386		95.082		pg/ml	
1,F1	Standard6	41.15	36.5	39.882		96.912		pg/ml	
1,F2	Standard6	41.15	39.5	42.339		102.884		pg/ml	
1,G1	Standard7	13.72	11.5	13.326		97.144		pg/ml	Extrapolated
1,G2	Standard7	13.72	12	14.165		103.261		pg/ml	

Loc	Sample ID	Net MFI	Test Result	Range	%CV of Replicates	Unit	Dilution Factor	Comments
1,A3	Unknown1	27.5	64.004			pg/ml	2.00	
1,A4	Unknown1	36	78.93			pg/ml	2.00	
1,A5	Unknown9	35.5	78.093			pg/ml	2.00	
1,A6	Unknown9	30.5	69.46			pg/ml	2.00	
1,B3	Unknown2	13	31.461			pg/ml	2.00	
1,B4	Unknown2	17	42.128			pg/ml	2.00	
1,B5	Unknown10	6.5	<13.717			pg/ml	2.00	
1,B6	Unknown10	5	<13.717			pg/ml	2.00	
1,C3	Unknown3	4	<13.717			pg/ml	2.00	
1,C4	Unknown3	11.5	26.651			pg/ml	2.00	Extrapolated
1,H1	Background0	-0.5					0.00	
1,H2	Background0	0.5					0.00	

13. The raw **.csv output file** is saved automatically to the xPONENT output folder (commonly located on the desktop).