Bio-Plex 200 Maintenance and SOP

User Guide and Logs

- User Guide (https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10022815.pdf)
- Printable Routine Maintenance Log (https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_4100205.pdf)

YouTube Videos

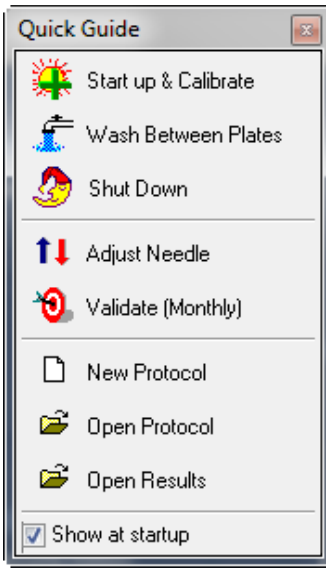
- Startup, Calibration, Program Method,
    - https://www.youtube.com/watch?v=6CD6W6RExRs
- Analysis
    - https://www.youtube.com/watch?v=2XqRUiq9w6Y

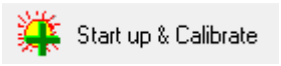
Instrument Warm-up / Startup

The Bio-Plex 200 requires 30 minutes for the lasers to warm. First thing in the morning turn on the Bio-Plex 200 and click the warm-up button (see below).

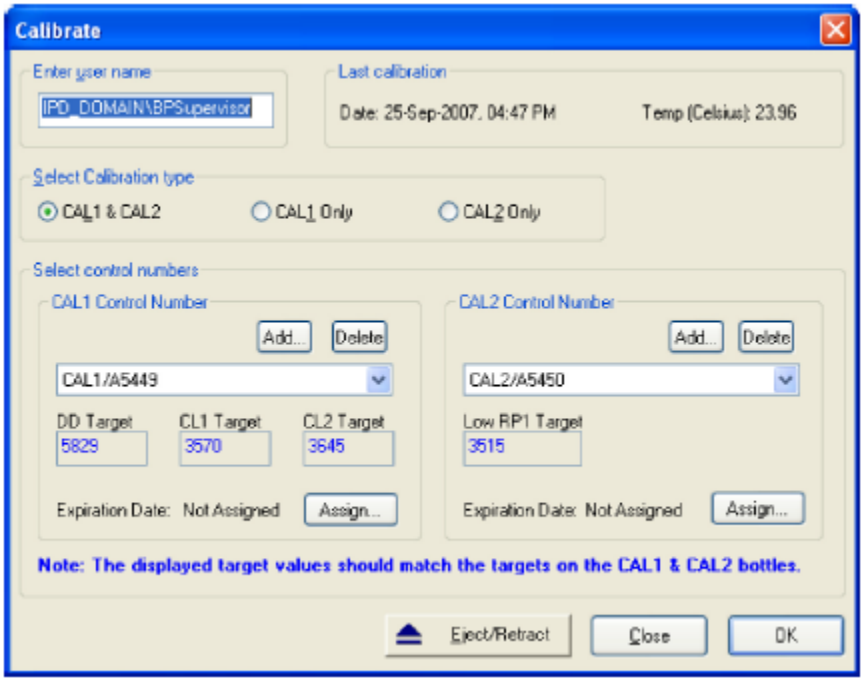
1. Turn on the Bio-Plex 200
    a. Turn on the HTF (power in back, lower right)
    b. Turn on the Reader (power in back, lower right)
    c. Turn on the Plate array (power in back, lower right)
    d. 

2. Ensure fluidics are ready
    a. Make sure you have enough sheath fluid for the day
    b. Empty waste container and make sure the lid is loose
3. Remove Calibration (And Validation Kit if required) Kit from refrigeration and allow to warm to room temperature.
4. Warm up Lasers
    a. Start Bio-Plex manager 
    b. Once connected, click the “Warm-Up” Icon 
    c. A timer will appear at the bottom
    d. Once warmed, a notice will appear on the screen. Click OK.
5. **<u>BEFORE THAWING ANY SAMPLES</u>**
    a. Perform Calibration
    b. If required perform Validation (follow request prompts)
6. Once Lasers are warm you can proceed with calibration, Proceed with Calibration
    a. Note the Quick Guide menu

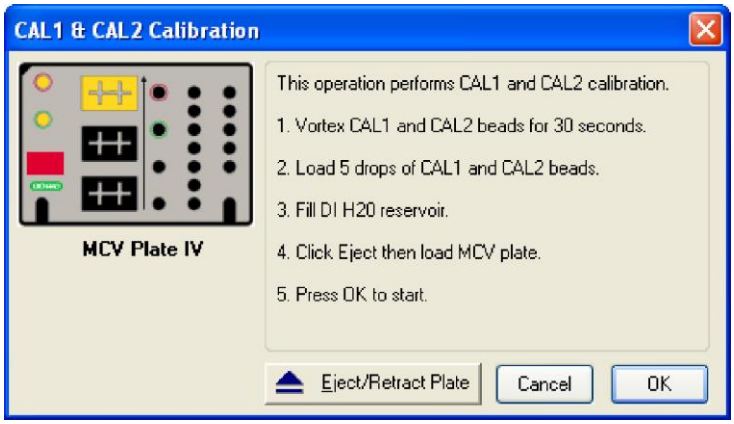
i. 

b. Click Startup and Calibrate 

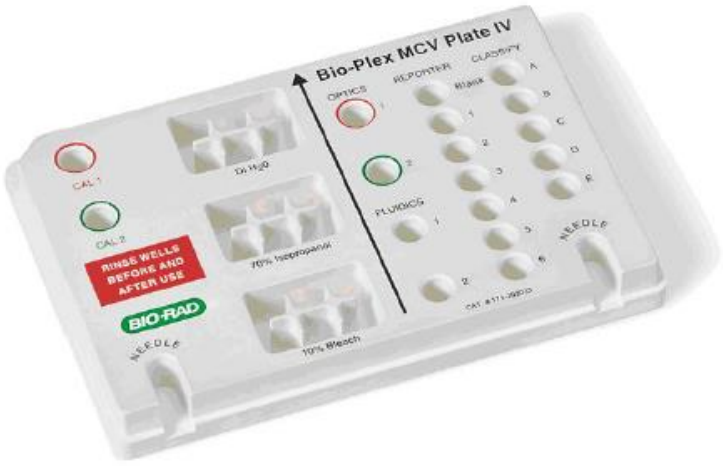
c. Follow the directions in the Startup and Calibrate Dialog Box for Preparing the MCV Plate
   i. The Dialog will look like this:
   ii. Ensure your control numbers are correct in the Calibrate dialog box, update if required

1. 

   iii. Click Ok

1. 

iv. Your plate looks like this:

1. 

v. When filling the Water / Alcohol / Bleach reservoirs, fill about 75% full.

vi. Click OK to run Calibration

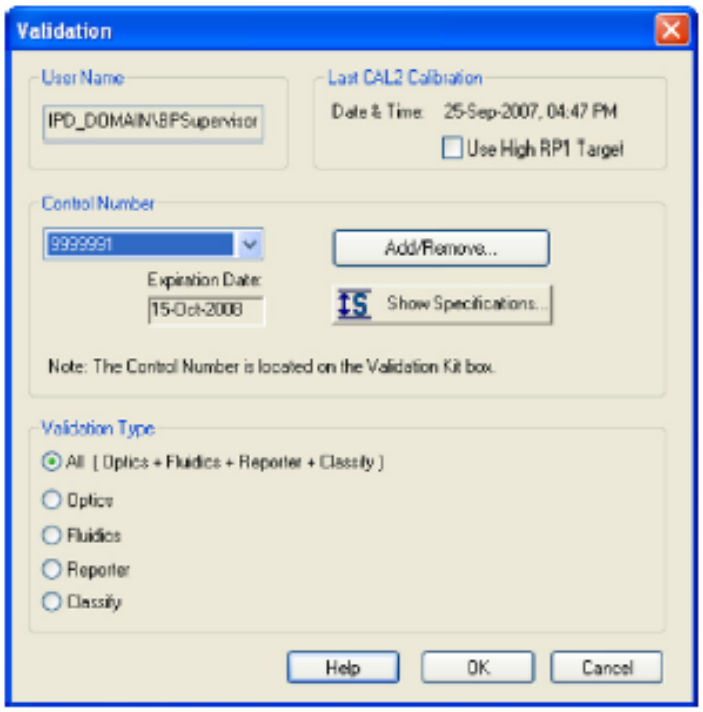
d. Once complete, it would display if the instrument passed or failed.

e. If passed and Validation is required, it will also notify you that Validation is Due in "X" days or Overdue. If overdue you can Start the calibration form this completion menu.

7. To perform Validation

a. Ensure the validation kit is listed in the Control number in the menu

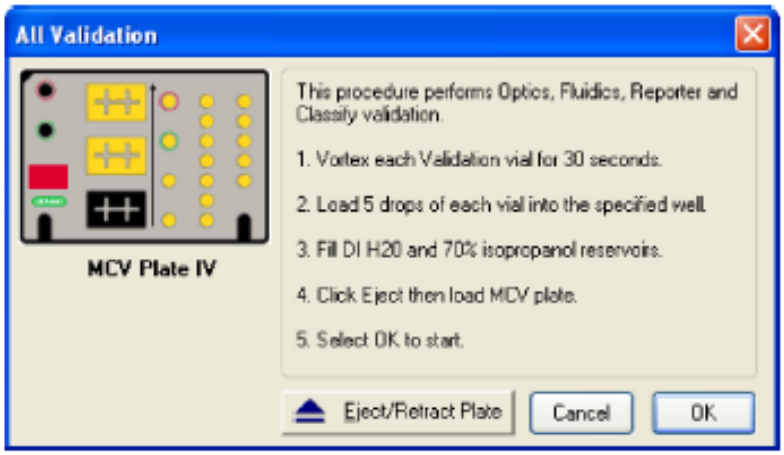
i.



b. Setup plate as prompted by the software

i. Vortex each tube

ii.



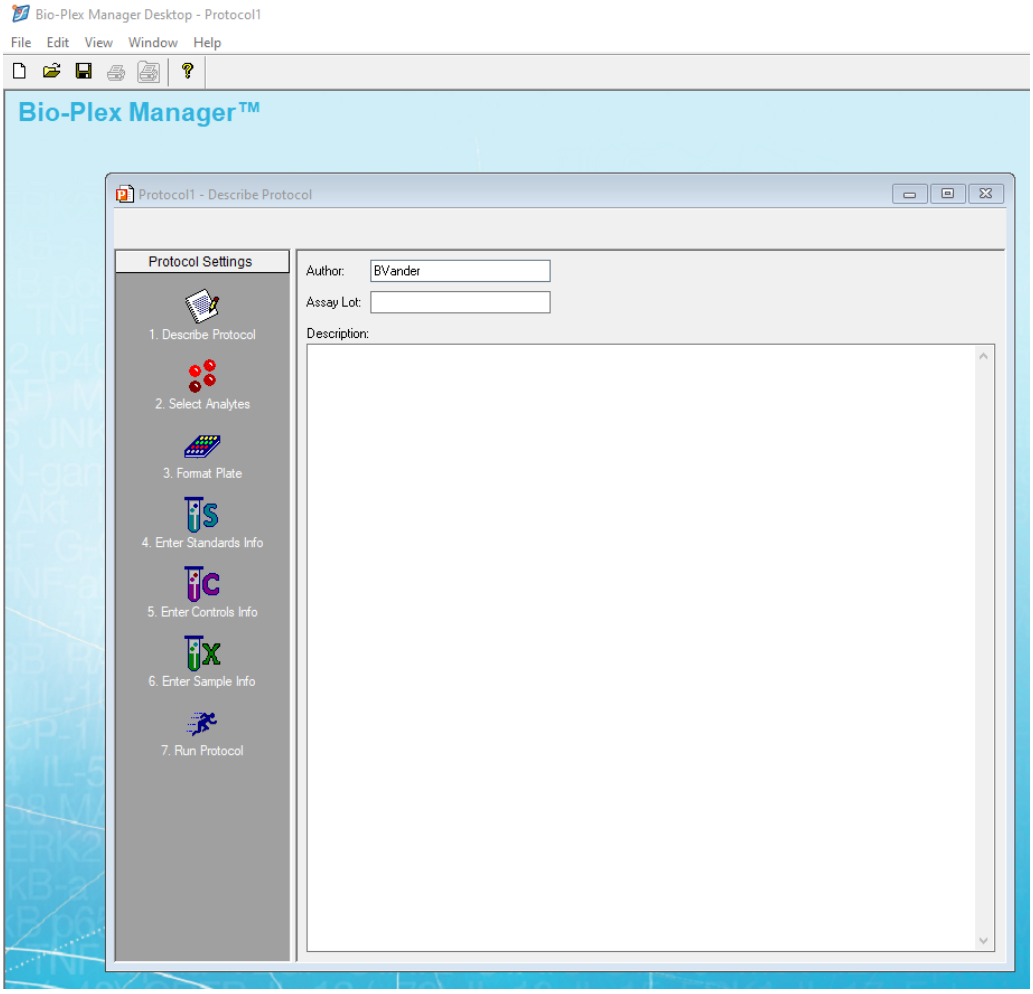
c. The validation kit tubes are arranged in a similar layout as the plate

Validation kit

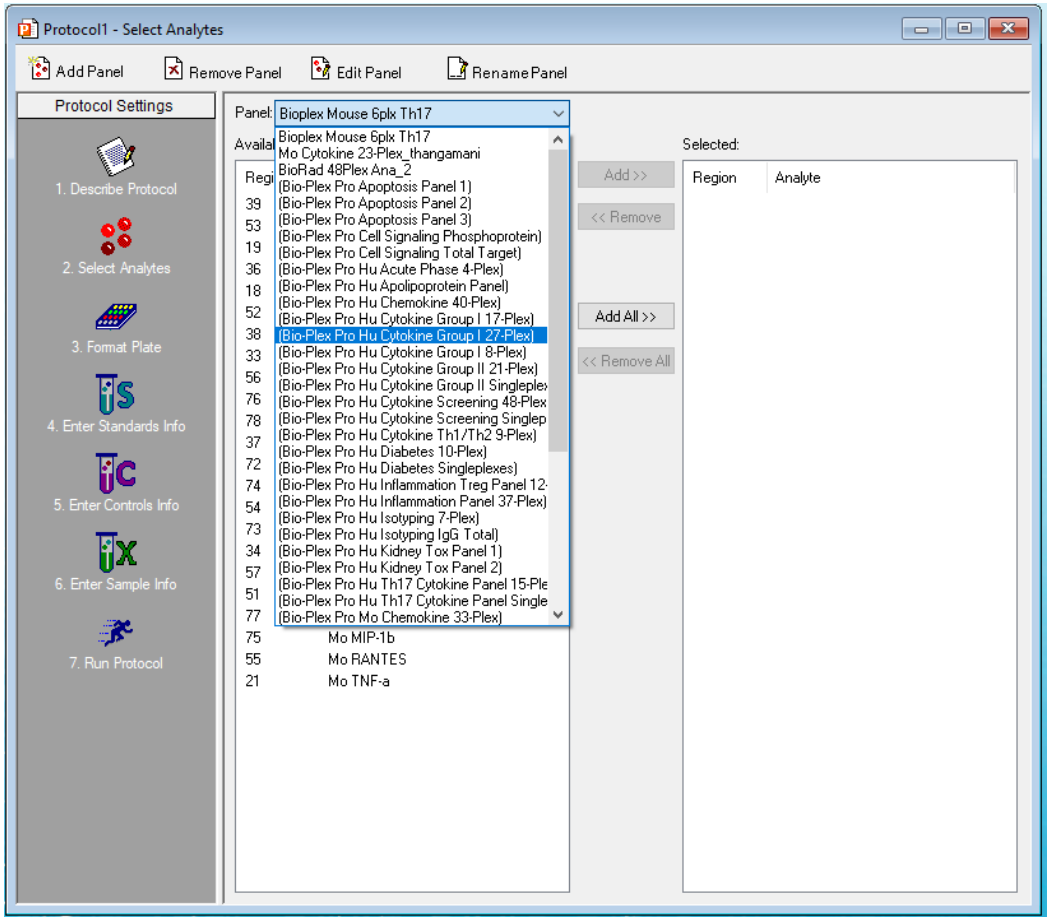
i.

d. Once Validation is complete and the instrument passes you are ready to run an assay

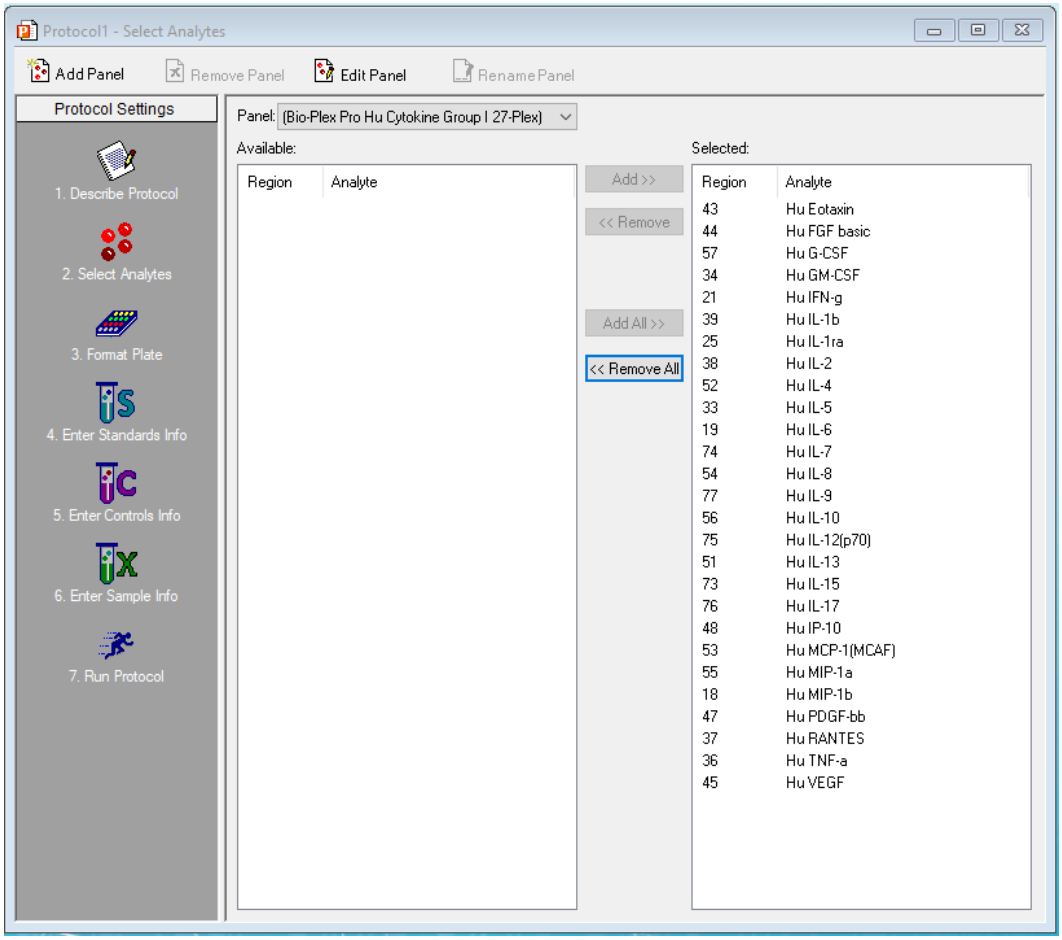
Programming a Protocol for Data Collection

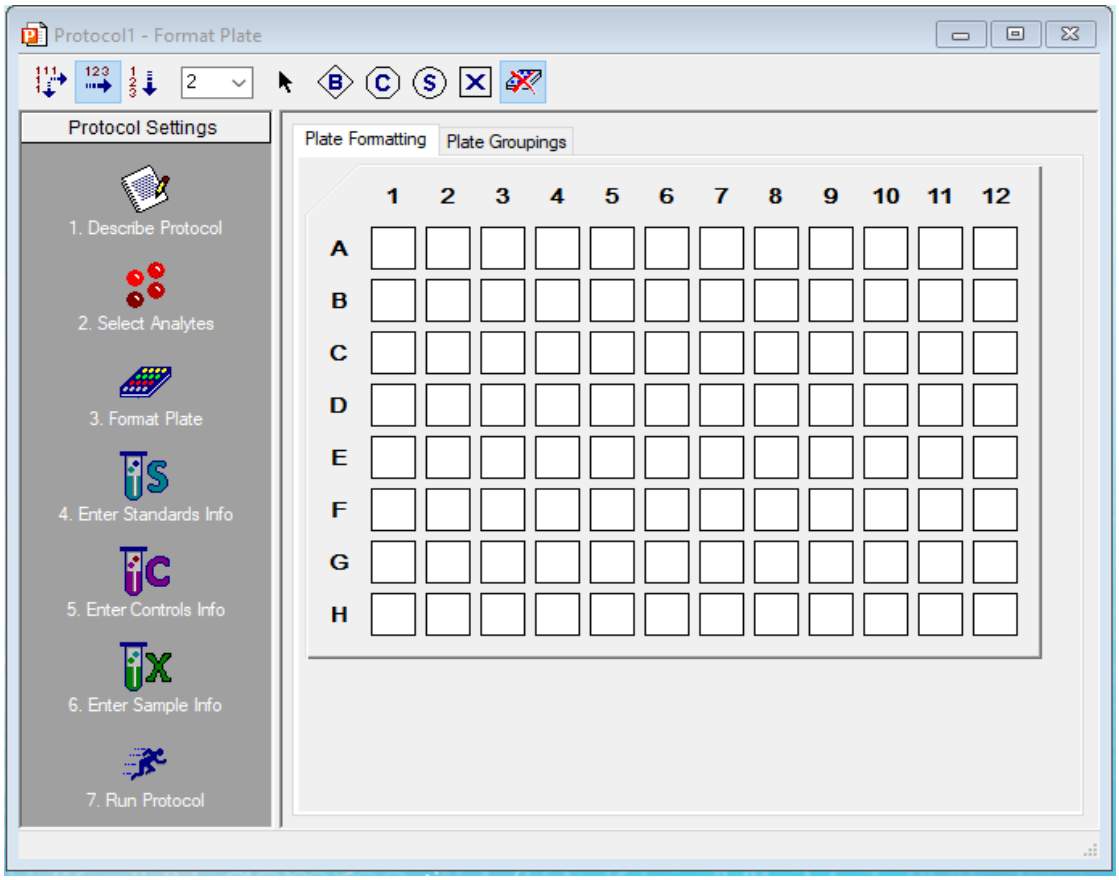
1. To create a new protocol, Click the New Protocol Button 
2. If running a pre-programmed protocol, Click the Open protocol button  and select the protocol you want to run.
3. Once the protocol is open, you will see the Protocol Window:
    a. 

4. You will program the protocol from top to bottom in numerical order
5. Describe Protocol
    a. Not required for a run, but recommended for notebook and record tracking

6. Select analytes
    a. In the drop-down menu, select the panel you will run
    b. 

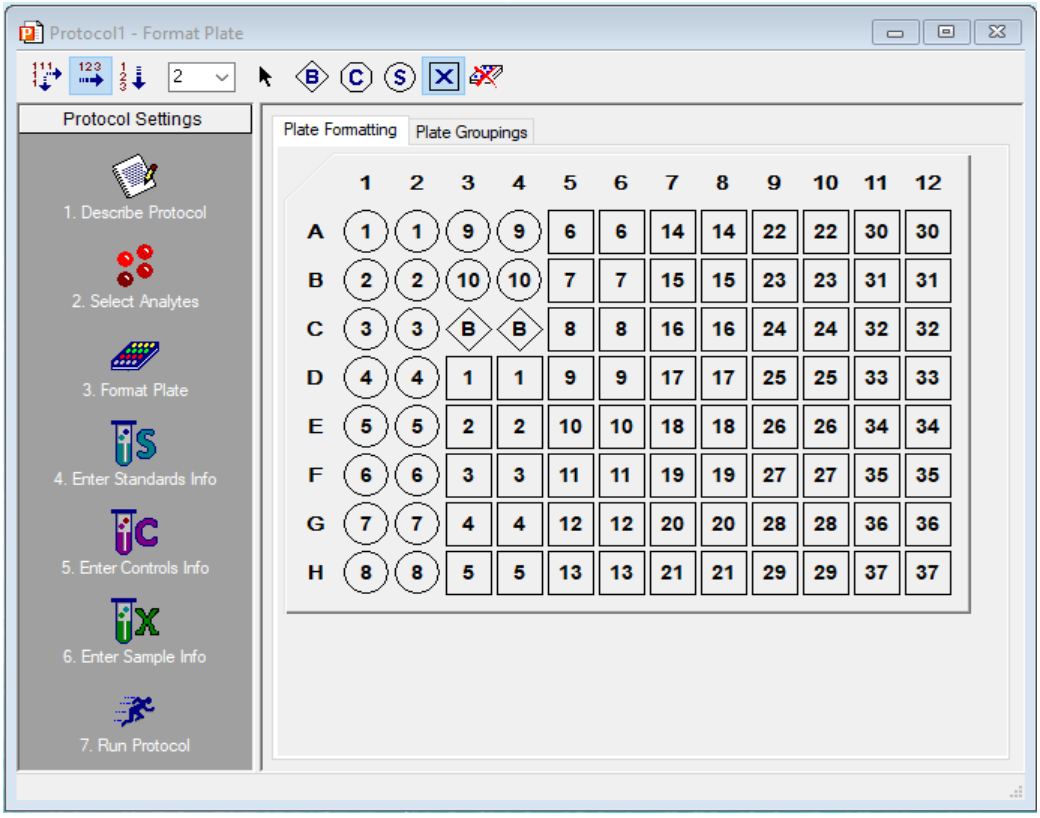
c. Click the “Add All >>” Button such that all the target analytes are listed on the left under “Selected:”

d. 

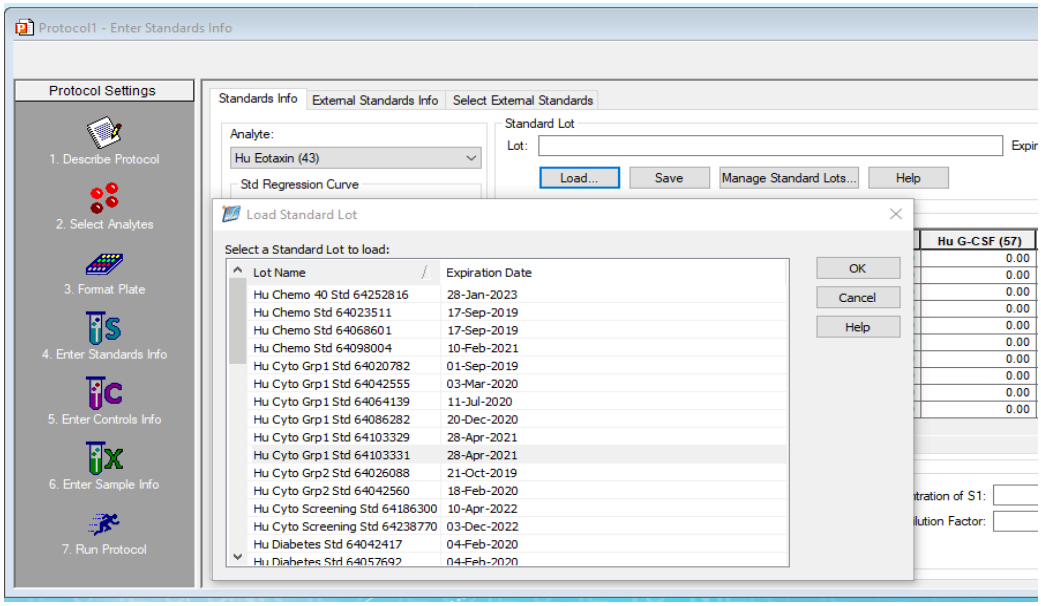
7. Format Plate
    a. Format Plate will look like this:
    b. 

    c. On the Top toolbar you will first select how you will fill the plate
        i. The arrow indicates the way you do replicates
        ii. The number indicates how many replicates
    d. You will then select the letter for the Type of fluid in each well, then select the well it is in or drag across a section
        i. B = Blank
        ii. C = Control
        iii. S = Standard
        iv. X = Unknown (your samples)
        v. Plate with red X is eraser tool

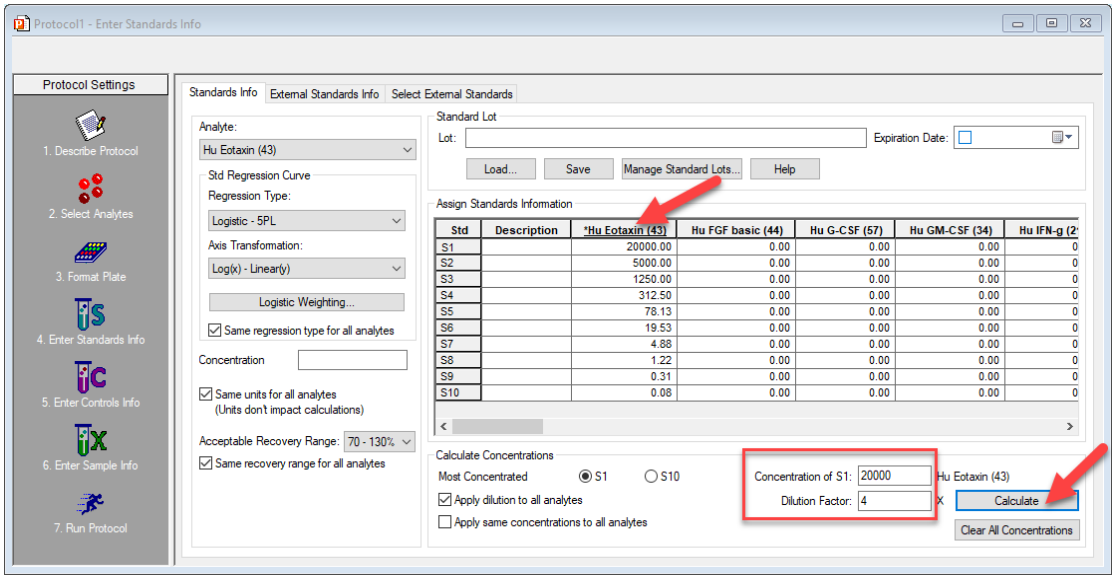
e. Your completed plate layout will look something like this

i. 

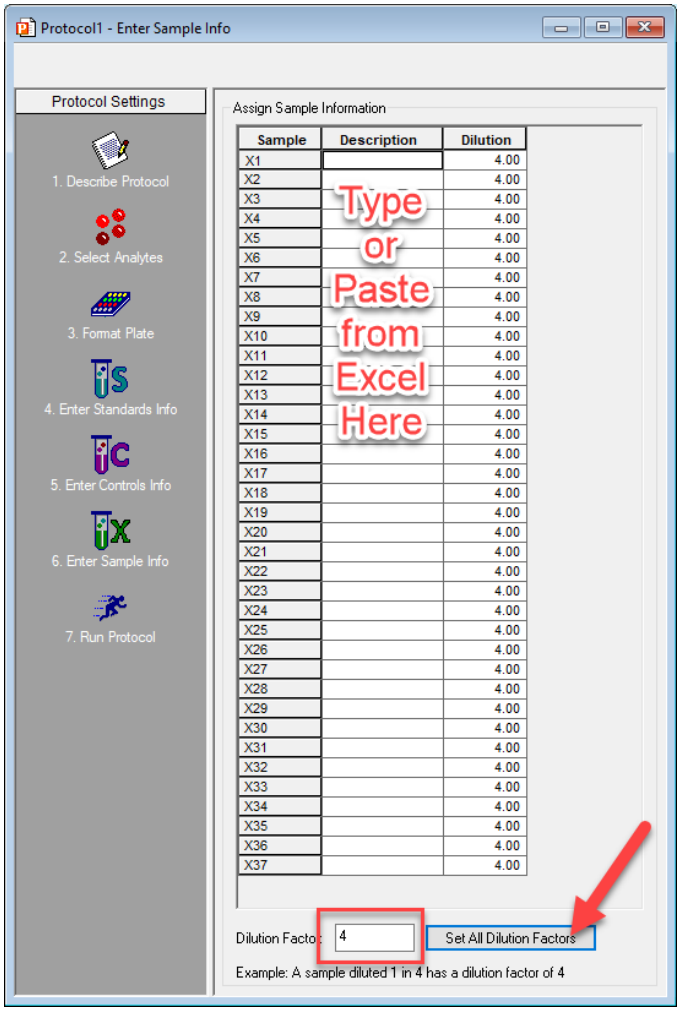
8. Enter Standards Info
    a. Ideally you will be able to import your standards info either from Bio-Rad or from a previous run (based on Standard Lot Number)
        i. Under Standard Lot Click the “Load…” Button, then select the Standard that matches yours
        ii.



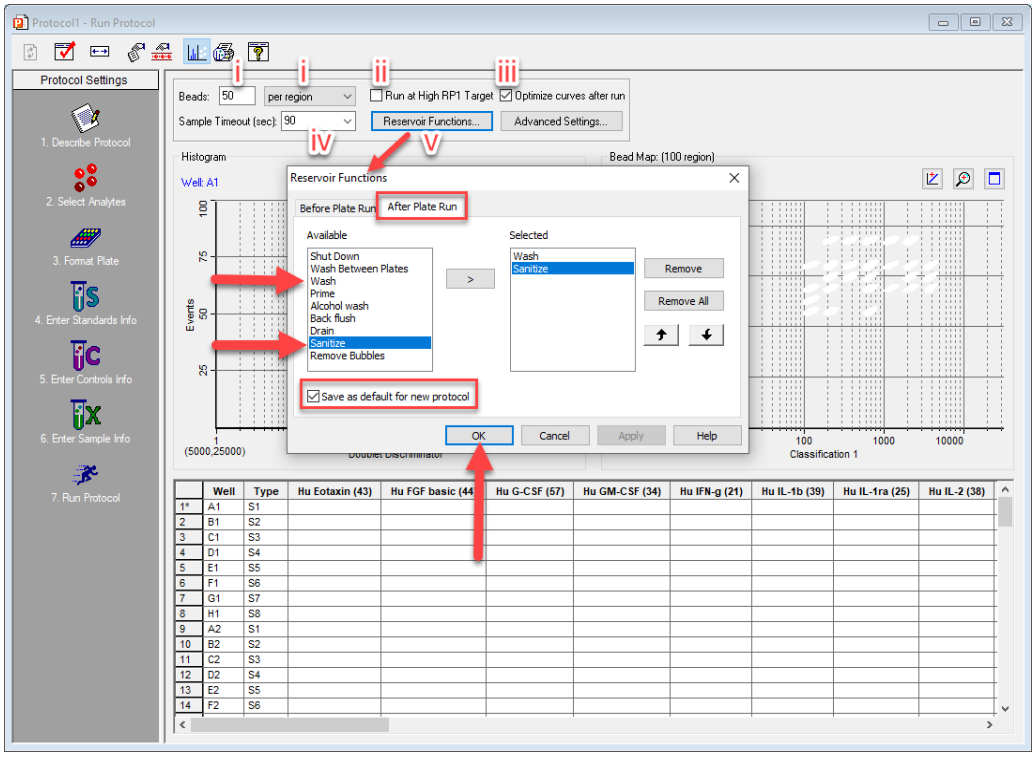
    b. Otherwise The standards will need to be entered in manually, then saved
        i. First, Find the standard values on the paper in the assay box. This is either on a yellow sticker OR a piece of 8.5x11 paper
        ii. In the Standards Info, click the top of the column of the analyte you want to enter
        iii. In the lower right of the screen enter the S1 concentration value for that analyte, then enter the dilution factor (4)
        iv. Click on Calculate
        v. Repeat ii -> iv for all analytes
        vi.



9. Enter Control Info
    a. If you have controls, Enter the concentrations in this area. Control information will be entered similarly to Standards and can also be found on an 8.5x11 sheet of paper.
10. Enter Sample Info
    a. Sample Descriptions can be typed or pasted from Excel
    b. You set sample dilution factors here; You can also set all dilutions by entering the dilution at the bottom and clicking “Set all Dilution Factors”.
        i. The Standard Dilution Factor for Plasma/Serum samples is 4
        ii.



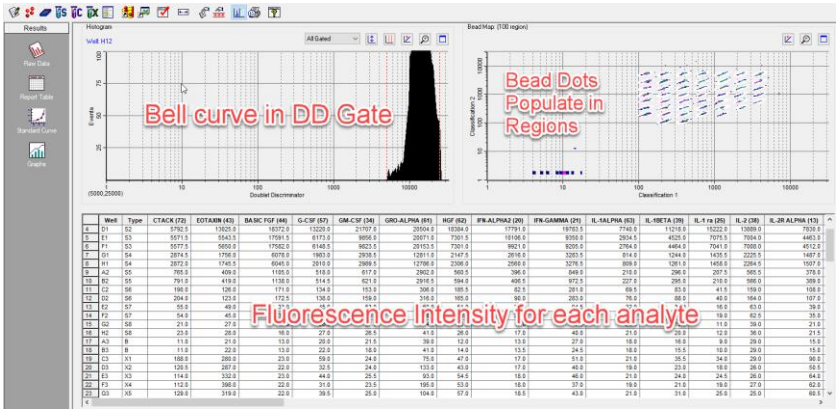
11. Run Protocol
    a. In this screen you will dictate the collection parameters of the hardware
        i. Beads = 50 per region
        ii. No High RP1 (Unless noted)
            1. Rat assays require high RP1
        iii. Do optimize curves after the run
        iv. Set a sample Timeout of 90 seconds
        v. Click Reservoir functions
            1. Click the “After Plate Run” Tab
            2. Select Wash then click “>”
            3. Select Sanitize then click “>”
            4. Check the box for “Save as Default for new protocol”
            5. Click OK
        vi.



    b. When connected to an instrument, the upper right will have a button that will allow you to start the run.
    c. Click the start run button
        i. It will open the tray, put in the Bio-Plex Assay plate as well as the reservoir

            1.
        ii. Bio-Plex manager will ask for a name for the run, this will be the filename.

d. During the Run, the Run screen will populate with data

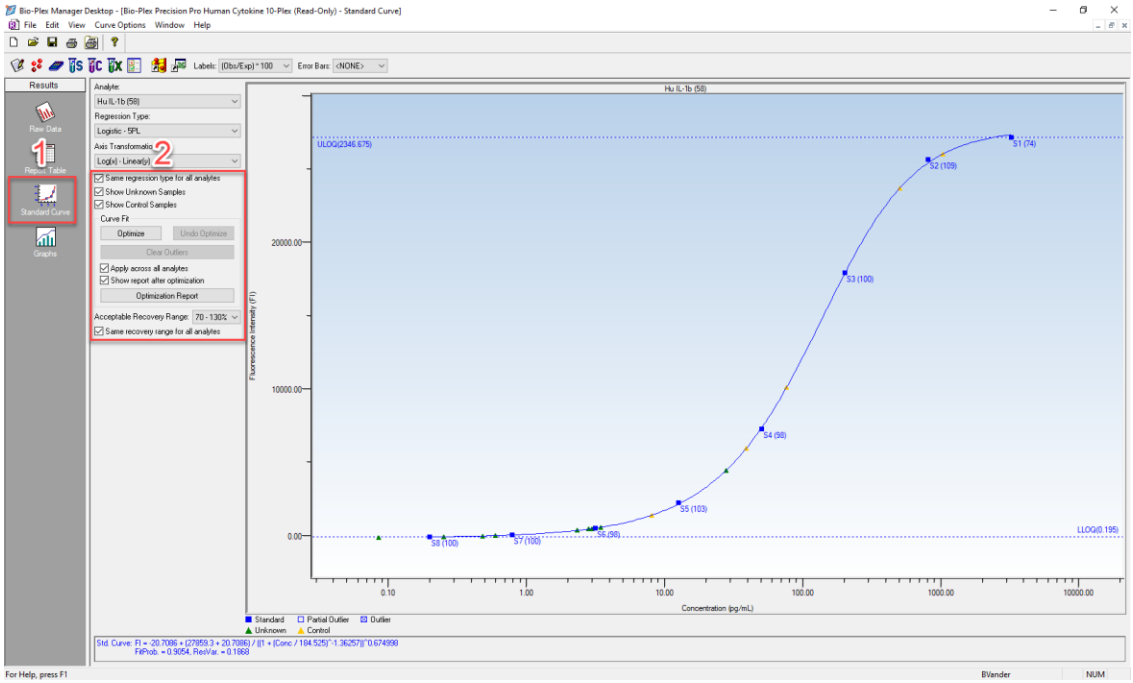


i.
ii. Upper left should display a “Bell-like” curve
iii. Upper right should populate with dots in each “cloud”. Each dot is a bead, and the “cloud” is the bead region.
iv. Bottom of the screen will show each analyte and the Fluorescence intensity for each analyte and each sample

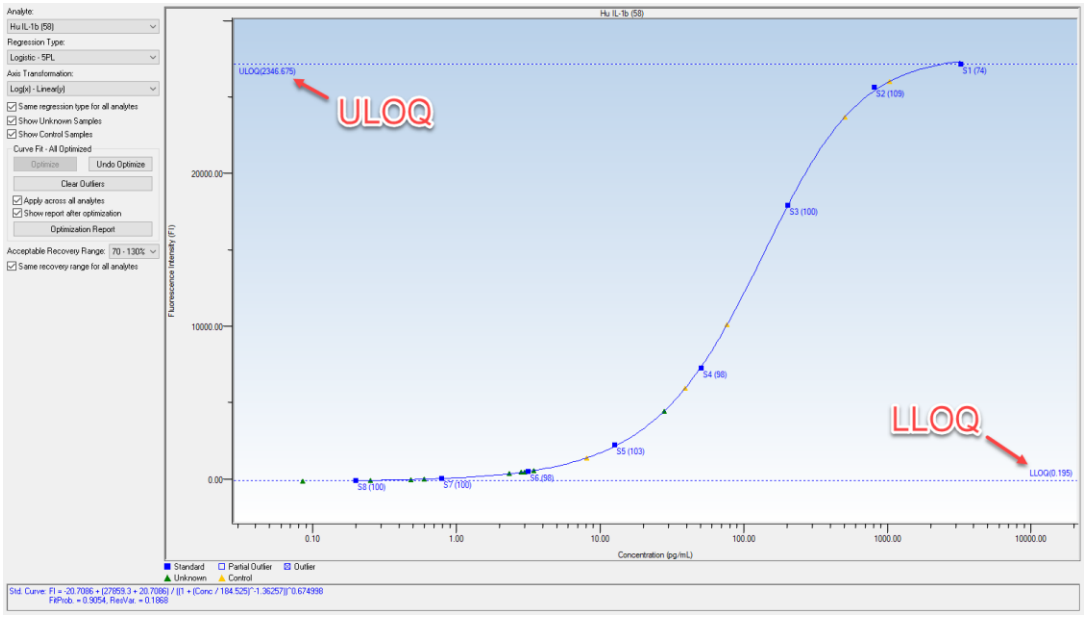
Rerun / Recovery Mode

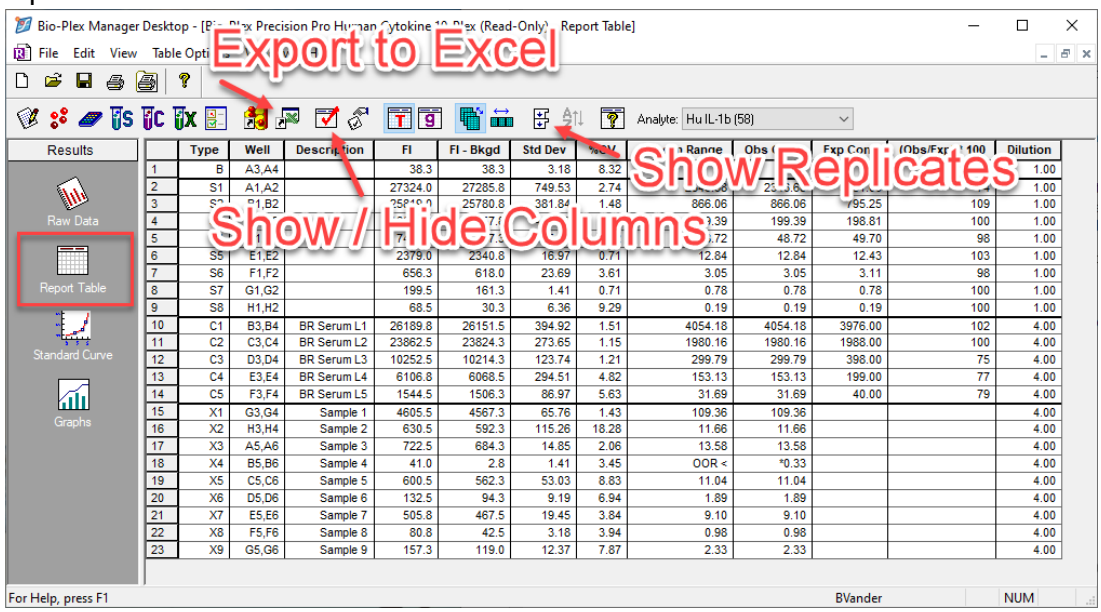
1. If there is a clog, you can stop the run and start from where you left off

Data Analysis

1. Open the file to be analyzed.
2. Once open, click on Standard Curve on the left side
    a. For regression type use Logistic - 5PL
        i. Note: For further reading see [Bulletin 3022]
    b. Ensure that the check boxes next to Same regression, show unknown, show control, apply across all analytes, show report after completion, and same recovery are all checked
    c. 

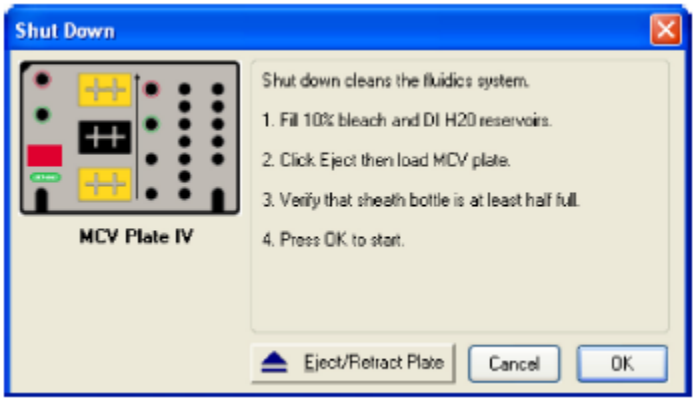
    d. Click optimize
    e. Once complete, you can review the report, then click close

3. Review each standard curve for fit
    a. Sometimes removing an S1 or S8/S9/S10 will help the overall fit
        i. To remove a standard curve point right click and hit “Select Outlier”
        ii. S1 is typically eliminated because of analyte saturation
        iii. Low concentrations are typically eliminated because of weak signal
    b. This graph shows the ULOQ (Upper Limit of Quantification) and LLOQ (Lower Limit of Quantification) in pg/ml
    c. 

4. Next, Click the “Report Table” button
    a. From here you can Export to Excel, Show or hide columns, and Show or hide replicates
    b. 

	Type	Well	Description	FI	FI - Bkgd	Std Dev	%CV	Obs Range	Obs Conc	Exp Conc	Obs/Exp	Dilution
1	B	A3,A4		38.3	38.3	3.18	8.32					1.00
2	S1	A1,A2		27324.0	27285.8	749.53	2.74					1.00
3	S2	B1,B2		25810.0	25780.8	381.84	1.48		866.06	866.06	795.25	109	1.00
4	S3	C1,C2		14	7.4				199.39	199.39	198.81	100	1.00
5	S4	D1,D2		7	7.1				48.72	48.72	49.70	98	1.00
6	S5	E1,E2		2379.0	2340.8	16.97	0.71		12.84	12.84	12.43	103	1.00
7	S6	F1,F2		656.3	618.0	23.69	3.61		3.05	3.05	3.11	98	1.00
8	S7	G1,G2		199.5	161.3	1.41	0.71		0.78	0.78	0.78	100	1.00
9	S8	H1,H2		68.5	30.3	6.36	9.29		0.19	0.19	0.19	100	1.00
10	C1	B3,B4	BR Serum L1	26189.8	26151.5	394.92	1.51		4054.18	4054.18	3976.00	102	4.00
11	C2	C3,C4	BR Serum L2	23862.5	23824.3	273.65	1.15		1980.16	1980.16	1988.00	100	4.00
12	C3	D3,D4	BR Serum L3	10252.5	10214.3	123.74	1.21		299.79	299.79	398.00	75	4.00
13	C4	E3,E4	BR Serum L4	6106.8	6068.5	294.51	4.82		153.13	153.13	199.00	77	4.00
14	C5	F3,F4	BR Serum L5	1544.5	1506.3	86.97	5.63		31.69	31.69	40.00	79	4.00
15	X1	G3,G4	Sample 1	4605.5	4567.3	65.76	1.43		109.36	109.36			4.00
16	X2	H3,H4	Sample 2	630.5	592.3	115.26	18.28		11.66	11.66			4.00
17	X3	A5,A6	Sample 3	722.5	684.3	14.85	2.06		13.58	13.58			4.00
18	X4	B5,B6	Sample 4	41.0	2.8	1.41	3.45		OOR <	*0.33			4.00
19	X5	C5,C6	Sample 5	600.5	562.3	53.03	8.83		11.04	11.04			4.00
20	X6	D5,D6	Sample 6	132.5	94.3	9.19	6.94		1.89	1.89			4.00
21	X7	E5,E6	Sample 7	505.8	467.5	19.45	3.84		9.10	9.10			4.00
22	X8	F5,F6	Sample 8	80.8	42.5	3.18	3.94		0.98	0.98			4.00
23	X9	G5,G6	Sample 9	157.3	119.0	12.37	7.87		2.33	2.33			4.00

Bio-Plex 200 Shutdown

1. Shut down procedures ensure the instrument is clean before powering off
2. To initiate the Shutdown procedure, click Shutdown 
3. Follow the on-screen instructions on how to prepare the MCV plate
    a. Water and 10% Bleach
    b. 

4. Allow the shutdown procedure to continue until complete (a dialog box will state Shutdown is complete)
5. Eject the MCV plate
    a. Rinse the MCV plate with DI water, place upside down on paper towels to dry
6. Turn off the switches to the Bio-Plex 200
    a. Turning off the hardware will cause a dialog box in Bio-Plex Manager to appear saying that it lost connection
    b. Turn off HTF
    c. Turn Off Reader
    d. Turn Off XY Plate platform
    e. 

Maintenance

Daily:

Start up, Calibrate, Wash Between Plates, Shutdown

Weekly:

Sonicate Needle, unclog, check for leaks

Monthly:

Run Validation, Clean exterior surface

Every 6 Months:

Replace syringe seal, clean ventilation filter

Yearly:

Replace sheath filter, replace air intake filter

Videos for Common Maintenance Tasks

Hardware Instruction Manual:
https://www.biorad.com/webroot/web/pdf/lsr/literature/10005042.pdf

Remove or replace Needle:
https://www.youtube.com/watch?v=o5jWPfVjYuk&t=51s

Adjust Probe Height:
https://www.youtube.com/watch?v=W-ckfD025jw

Troubleshooting Guide

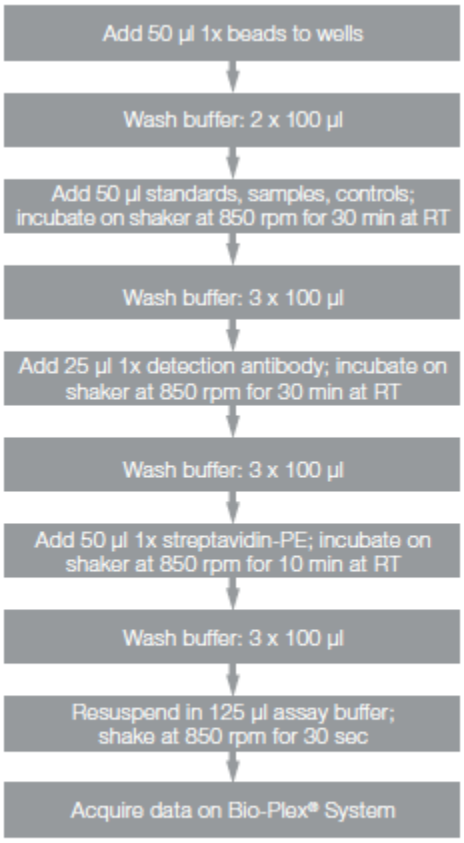
This troubleshooting guide addresses problems that may be encountered with Bio-Plex Pro™ Assays. If you experience any of the problems listed below, review the possible causes and solutions provided. Poor assay performance may also be due to the Bio-Plex® Suspension Array Reader. To eliminate this possibility, use the validation kit to determine whether the array reader is functioning properly.

Problem and Possible Causes	Possible Solutions
High Inter-Assay Coefficient of Variation (CV)	
Standards and controls were not reconstituted consistently between assays	Incubate the reconstituted standards for 30 min on ice. Always be consistent with the incubation time and temperature
High Intra-Assay CV	
Improper pipetting technique	Pipet carefully when adding standards, controls, samples, detection antibodies, and streptavidin-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer
Reagents and assay components not equilibrated to room temperature prior to pipetting	All reagents and assay components should be equilibrated to room temperature prior to pipetting
Contamination with wash buffer during wash steps	During the wash steps, be careful not to splash wash buffer from one well to another. Be sure to monitor residual volume after each wash cycle. Ensure that the microplate shaker setting is not too high. Reduce the microplate shaker speed to minimize splashing
Slow pipetting of samples and reagents across the plate	Sample pipetting across the entire plate should take less than 4 min. Reagent pipetting across the entire plate should take less than 1 min
Bio-Plex Wash Station: insufficient washing due to clogged pins	Clean dispensing pins with the thicker of the two cleaning needles provided with washer. Perform regular rinses to minimize salt buildup
Low Bead Count	
Miscalculation of bead dilution	Check your calculations and be careful to add the correct volumes
Beads clumped in multiplex bead stock tube	Vortex for 30 sec at medium speed before aliquoting beads
Assay plate not shaken enough during incubation steps and prior to reading	Shake the plate at 850 ± 50 rpm during incubation steps and for 30 sec immediately before reading the plate
Reader is clogged	Refer to the troubleshooting guide in the Bio-Plex 200 System hardware instruction manual (document #10005042)
Incorrect needle height of the reader	Adjust the needle height to coincide with the plate type provided in the kit
Low Signal or Poor Sensitivity	
Standards reconstituted incorrectly	Follow the standard preparation instructions carefully
Detection antibody or streptavidin-PE diluted incorrectly	Check your calculations and be careful to add the correct volumes
High Background Signal	
Incorrect buffer was used (for example, assay buffer used to dilute standards)	Use standard diluent to dilute standards and as a reagent blank
Accidentally spiked blank wells	Do not add any antigens to the blank wells
Detection antibodies or streptavidin-PE incubated too long	Follow the procedure incubation time precisely

Problem and Possible Causes	Possible Solutions
Poor Recovery	
Expired Bio-Plex reagents were used	Check that reagents have not expired. Use new or nonexpired components
Incorrect amounts of components were added	Check your calculations and be careful to add the correct volumes
Microplate shaker set to an incorrect speed	Check the microplate shaker speed and use the recommended setting. Setting the speed too high may cause splashing and contamination. Setting the speed too low may cause low assay signal and false plateau or saturation at the high end of standard curves. Use the recommended plate shaker
Quality controls do not fall within expected ranges	Make sure that the control vial is reconstituted at the same time as standards and in the same standard diluent HB. Incubate for precisely 30 min
Improper pipetting technique	Pipet carefully when adding standards, samples, detection antibodies, and streptavidin-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer
Impact of Sample Matrix	
Poor precision in serum and plasma sample measurements	Check whether any interfering components, additives, or gel from separators were introduced into the samples. Avoid using hemolyzed and heavily lipemic samples. Remove visible particulate in samples by centrifugation. Avoid multiple freeze-thaw cycles of samples

Running a Bio-Rad Bio-Plex Assay

Assay Workflow



Note: Once thawed, keep samples on ice. Prepare dilutions just prior to the start of the assay and equilibrate to room temperature before use.

Assay Steps

1. When Calibration and Performance verification passes prepare samples and workspace.
    a. Thaw on ice
    b. Vortex well
    c. Centrifuge at 10,000 X g @ 4°C for 8 minutes, then keep samples at 4°C.
        *i. (**note**: when performing 2 plates per day, thaw, vortex and spin all samples prior to start of assay; when the first spin is ongoing, prepare STD and put SAMPLE DILUENT into a round-bottomed 96well plate)*
2. Label tubes:
    a. Eppendorf for Standards, Blank,
    b. 15ml for Beads, Detection antibody, SA-PE
3. To the bead dilution tube, detection antibody dilution tube and SA-PE dilution tube, you can go ahead and add the correct volume of reagent-specific buffer.
    a. Bead diluent: 5130uL Assay Buffer
    b. Detection Antibody diluent: 2700uL Detection Antibody Diluent HB
    c. SA-PE diluent: 5940uL Assay Buffer
4. Resuspend Standard and Control:
    a. Add 250 µl standard diluent HB to standard vial, vortex and store on ice for exactly 30 minutes, mixing by inversion intermittently
    b. Add 250 ul standard diluent HB to control vial, vortex and store on ice for exactly 30 minutes, mixing by inversion intermittently
        i. Standard and Control must be used during this assay, do not freeze for future runs.
5. Prepare Samples:
    a. During 30-minute Standard/Control incubation, add 150 µl of sample diluent to round bottomed 96well plate where samples will be diluted (according to Plate Layout)
    b. <u>(If Applicable) Transfer 96well plate to Biological Safety Cabinet, Class 2</u>
    c. Add 50 µl of plasma sample (1:4 dilution) according to Plate Layout. Mix gently with pipette and store on ice until time to begin assay.

6. Prepare Standards:
    a. During 30-minute Standard incubation, label 10 Eppendorf tubes S1-S10 and Blank. (When 2 plates/day assayed, use 1 Standard vial only). Store on ice until use.
    b. Add 150ul Standard diluent to tubes S2 - S10 and Blank
        i. Do not add Diluent to S1
    c. Adding Standard to tubes:

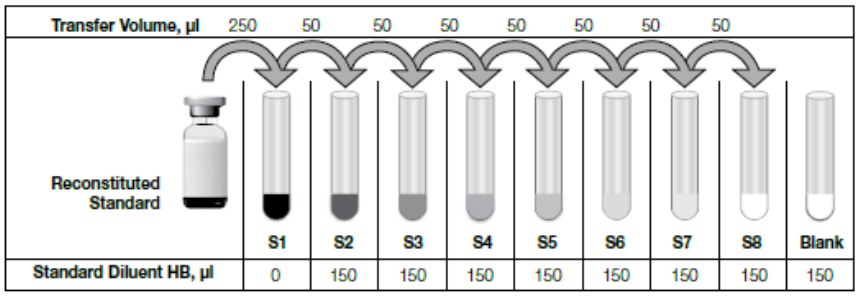


Fig. 3. Preparing a fourfold dilution series with a single reconstituted standard.

Note: For samples with very low endogenous analytes, preparing an additional standard point to extend the bottom end of the standard curve may help to improve sample detectability.

        i. For S1: add 250 µl from Standard vial.
        ii. For S2: transfer 50 µl from S1 to S2, vortex 5 seconds to mix.
        iii. For S3: transfer 50 µl from S2 to S3, vortex 5 seconds to mix.
        iv. For S4: transfer 50 µl from S3 to S4, vortex 5 seconds to mix.
        v. For S5: transfer 50 µl from S4 to S5, vortex 5 seconds to mix.
        vi. For S6: transfer 50 µl from S5 to S6, vortex 5 seconds to mix.
        vii. For S7: transfer 50 µl from S6 to S7, vortex 5 seconds to mix.
        viii. For S8: transfer 50 µl from S7 to S8, vortex 5 seconds to mix.
        ix. For S9: transfer 50 µl from S8 to S9, vortex 5 seconds to mix.
        x. For S10: transfer 50 µl from S9 to S10, vortex 5 seconds to mix.
7. Preparing Beads:
    a. Dilute beads in 15 ml conical tube:
        i. Note: premixed assay vs single-plex assays differ
    b. Transfer 570 µl of beads (vortexed well, transferring 200 µl, 200 µl, and 170 µl of well-mixed beads) and to the 5130 µl of Assay Buffer.
    c. Vortex and keep from light.
8. If Standard and Control are still incubating STOP HERE and Wait.
    a. When the Standards, Controls, and Samples are all ready to be put into the assay plate you may proceed.

9. Adding Beads to the Assay Plate:
    a. Clamp assay plate on magnetic holder.
    b. Add 50 µl of 1X beads to all wells.
10. Wash beads on plate washer using the MAGX2 setting.
    a. (If Applicable) Transfer assay plate to BSL hood.
    b. Program -> Magx2 -> Enter until it starts.
    c. It will pause for about 30 seconds before starting.
11. Remove plate after completion.
    a. This contains beads with very little buffer. Work quickly, carefully, and diligently to transfer your sample into the plate. ACCURACY IS CRITICAL, DO NOT RUSH!
12. **(Under the Biological Safety Cabinet Class 2)**, transfer 50 µl of Standards and Samples (diluted) from 96well plate onto assay plate using a multichannel pipette (according to plate map layout).
13. Cover plate with foil seal, make sure to press seal down so that all wells are separate.
    a. Shake on rotator at 850 rpm for 30 minutes.
    b. Start a timer for 20 and 30 minutes! Keeping a consistent assay time is critical for multiple-assay experiments
14. After 20 minutes have passed, mix and quick spin Detection antibody (10X);
    a. add 300 µl of Detection antibody to 2700uL of Detection antibody diluent. Mix well.
        i. Note: premixed assay vs single-plex assays differ
15. After 30-minute incubation is complete, Turn off plate shaker
16. Wash plate on plate washer under the BSCII and using the MAGx3 setting.
    a. Programs -> Magx3 -> enter until wash
17. Remove assay plate after completion.
18. Vortex Detection antibody (1X); add 25 µl to each well.
19. Cover plate with sealing foil.
    a. Shake at 850 rpm for 30 minutes.
    b. Set a timer for 20 minutes and 30 minutes.
    c. NOTE: IF running 2 plates, time to start 2nd plate;
        i. record Detection antibody incubation time to also incubate 2nd plate Detection antibody incubation the same exact time.
    d. Click the Laser Warm up button again to reset the 4-hour timer.

20. After 20 minutes of Detection antibody incubation (or after 2nd plate has been started), Prepare SA-PE
    a. vortex and quick spin 100X SA-PE.
    b. Add 60 µl (100X) SA-PE to 5940uL of Assay Buffer.
    c. Vortex and protect from light (Cover with foil or put in drawer)
21. After 30-minute Detection antibody incubation, turn off plate shaker
    a. wash 1st plate on plate washer, setting MAGX3.
    b. Remove afterwards
22. Vortex 1X SA-PE and add 50 µl to each well.
23. Cover with foil seal
24. shake on rotator at 850 rpm for 10 minutes.
    a. Exceeding 10 minutes will increase background. Set a timer
        i. Note: Wait at assay, do not become distracted.
25. After 10-minute Detection antibody incubation, turn off plate shaker
    a. Wash plate on plate washer, setting MAGX3.
    b. Remove afterwards
26. Resuspend all wells with 125 µl of Assay Buffer.
27. Cover with foil cover and shake for a full 30 seconds.
28. Open Bio-Plex Manager 6.2
    a. If running a new program, start from the beginning of the next section
    b. If running a pre-saved program, open and start form step 11.