

Configuring the SpectraMax[®] i3x Multi-Mode Microplate Reader for ValitaTiter Assays

Setup Guide



Configuring the SpectraMax i3x Multi-Mode Microplate Reader for ValitaTiter Assays

The SpectraMax i3x Multi-Mode Microplate Reader has been validated for use with the ValitaTiter and ValitaTiter Plus assays. The following detection cartridge is required:

Fluorescence Polarization (FP-FLUO) Detection Cartridge (Molecular Devices cat. #0200-7009)

Optimal instrument settings for ValitaTiter (P/N VAL003 or VAL013, left table) and ValitaTiter Plus (P/N VAL004 or VAL014, right table) assays on the SpectraMax i3x reader are shown below. Read height should be optimized for each new assay. Microplate optimization should be performed the first time the assay is run, and subsequently for each new lot of plates, to ensure that the center of each well is read.

ValitaTiter settings

Parameter	Setting
Optical Configuration	FP-FLUO
Read Mode	FP
Read Type	Endpoint
Excitation	485 nm
Emission	535 nm
Plate Type	96-well: 96 Well Corning Half Area (must be optimized*)
G Factor	1.0
Integration time	400 ms
Read height (mm)	Optimize*
G factor (set in Data Reduction)	1.0

ValitaTiter Plus settings

valitariter i las settings	
Parameter	Setting
Optical Configuration	FP-FLUO
Read Mode	FP
Read Type	Endpoint
Excitation	485 nm
Emission	535 nm
Plate Type	96-well: 96 Well Corning Half Area (must be optimized*)
G Factor	1.0
Integration time	400 ms
Read height (mm)	Optimize*
G factor (set in Data Reduction)	1.0

*For more information, please see the section "Microplate & read height optimization" below.

Setting up standard curves for ValitaTiter and ValitaTiter Plus assays

The following is an example of how standard curves for the ValitaTiter assays can be set up and read on a SpectraMax i3x reader.

Materials

- ValitaTiter (Molecular Devices cat. #VAL003 or VAL013) or ValitaTiter Plus (Molecular Devices cat. #VAL004 or VAL014) 96-well assay plate
- Human IgG for use as standard (e.g., Sigma cat. #12511)
- Assay diluent may be cell culture medium
- Note: this setup guide uses human IgG from serum as a standard, but when running your assay, you should select a standard that is as similar as possible to your test samples (e.g., same IgG isotype) in order to ensure accurate results.

Before starting, warm the ValitaTiter plate, assay diluent, and IgG stock solution to room temperature.

Standard curve setup

Make an 8-point standard curve starting at the highest recommended concentration for the assay (100 μ g/mL for ValitaTiter, or 2000 μ g/mL for ValitaTiter Plus) and including a standard with no IgG (standard 8, 0 μ g/mL).

You will need enough of each standard concentration for 60 μ L/well in triplicate wells per standard concentration, or 200 μ L per standard concentration.

- ValitaTiter: Make up 600 μ L of standard 1 at 100 μ g/mL in assay diluent.
- ValitaTiter Plus: Make up 700 μL of standard 1 at 2000 μg/mL in assay diluent.

Make standards by following the tables below. Standards may be made in microfuge tubes, or in a multichannel reagent basin for ease of pipetting into the 96-well plate.

ValitaTiter

Standard	Concentration (µg/mL)	Standard 1 (µL)	Diluent (µL)	Assay plate wells
1	100	200	0	A1–A3
2	80	160	40	B1–B3
3	40	80	120	C1–C3
4	20	40	160	D1–D3
5	10	20	180	E1–E3
6	5.0	10	190	F1–F3
7	2.5	5	195	G1–G3
8	0	0	200	H1–H3

ValitaTiter Plus

Standard	Concentration (µg/mL)	Standard 1 (µL)	Diluent (µL)	Assay plate wells
1	2000	200	0	A1–A3
2	1600	160	40	B1–B3
3	1200	120	80	C1–C3
4	800	80	120	D1–D3
5	400	40	160	E1–E3
6	200	20	180	F1–F3
7	100	10	190	G1–G3
8	0	0	200	H1–H3

Set up assay plate

- 1. Pipet 60 µL diluent to each assay well. Use reverse pipetting to avoid bubbles, and do not mix yet.
- 2. Pipet 60 μ L standard to triplicate assay wells containing diluent. Do not mix yet.
- 3. After all standards are added to wells, mix contents of wells very gently by pipetting up and down 3X using a multi-channel pipettor. Avoid introducing any bubbles.
- 4. Incubate at room temperature for 5 minutes (ValitaTiter) or 15 minutes (ValitaTiter Plus).
- 5. Read the plate on the SpectraMax i3x reader equipped with a Fluorescence Polarization (FP-FLUO) Detection Cartridge and using the optimized settings shown above.

Microplate & read height optimization

Microplate optimization ensures that the center of each well of the microplate is detected, providing more optimal results. This optimization should be run for each microplate reader, detection cartridge, or optical configuration (e.g., FP filters) used, as well as with each new lot of assay plates.

Read height optimization ensures that maximal signal is detected from the assay wells. It can be run for each new assay set up, but it need not be re-run as long as the plate type, plate lot number, and assay volume remain the same.

For microplate optimization, make sure all four corner wells (A1, H1, A12, and H12) contain material that will produce a fluorescent signal – for the ValitaTiter plates, assay wells containing diluent and sample, or wells containing diluent, are sufficient.

To perform microplate and read height optimization, make sure the box "Show Pre-Read Optimization Options" in the Settings dialog box in SoftMax Pro Software is checked:

More Settings		
Read Order	Row	~
Show Pre-Read Optimization Options		

When you are ready to read the plate, click "Read", and the following dialog box will appear:

Pre-Read Optimization Options	1	×
Optimization Options		-
 Run Microplate Optimizatio the measurement accuracy, the microplate lot changes. 		
 Run Read Height Adjustmer the measurement accuracy, the volume changes. Curren 	run Read Height Adjustme	nt each time
	Run Optimization	
Microplate Options		
	Plate is Lidded	
	Read Plate	Cancel

Make sure both boxes are checked, and then click "Run Optimization".

Follow the instructions that appear:

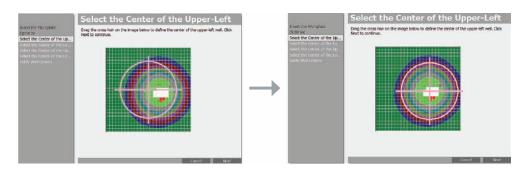
rner we	lls will b	e sc	anne	d to	opti	mize	the	mic	ropl	ste.	Click	Ne	e to	cont	inue.					samp	oles in
G	Open	the	Micr	opla	te Di	rawe	1 23						he M Iscap		olate	Orie	entat	tion			
	Close	e the	Micr	opla	ite D	rawe	er :														
1	2	1	3		4	1	5	1	6	1	7	1	8	1	9	L	10	1	11	1	12
																				(
-																					
1																					

Click "Next", and the reader will start scanning the four corner wells; a progress indicator will appear:

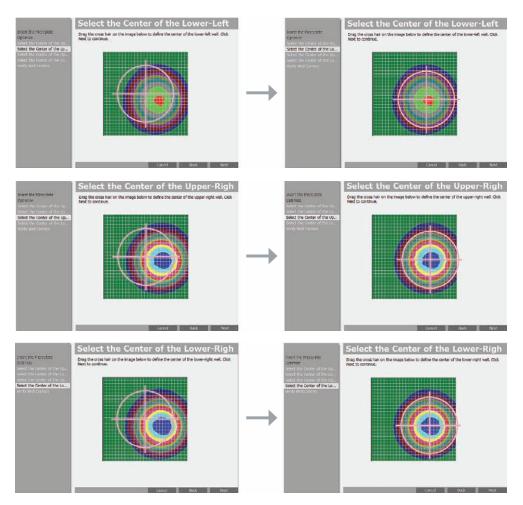
Well Scan in Prog	gress		
Please wait while the wells are scanned	for optimization.		
Scanning wells for optimization			
Elapsed Time	00:06:29		
Press Cancel Reading To StopLaby Optimization	sare Stop Optimization		
		Cancel	Net

After the reader finishes scanning the four corner wells, you will see a screen with an image of the upper left corner well and a pink crosshair. Click on the crosshair and move it so that it is centered over the well image. Make sure to align the crosshair to the outermost ring, or overall ring pattern, but not necessarily to the centermost spot.

Here is an example of the initial image, followed by the crosshair-adjusted image:



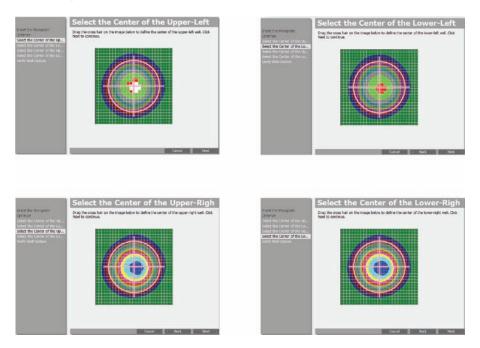
Click 'Next' and repeat for the remaining three corner wells:



Save the optimized microplate via the dialog box that appears. Note that a bracketed addition is made to the original microplate name to indicate that it has been optimized. Here you may also change the name of the plate:

	oplate definition. Microplate Dimensions	
	Bottom-row y offset (mm)	10.814
	Column spacing (mm)	9.028
	Left-column x offset (mm)	13,995
	Right-column x offset (mm)	12.46
	Row spacing (mm)	9.033
	Top-row y offset (mm)	11.355
,	Microplate Name	
	Microplate Name	96 Well Corning Half Area opague [MONO-Landscap
	ttom-row y offset (mm) e distance in millimeters from the lowe	r edge of the microplate to the horizontal center of the bottom row

The new, optimized microplate dimensions will now ensure that each well is read in its center. Thus, if the microplate optimization were re-run, the images would now appear centered and would require no further adjustment:



Next, perform read height optimization. First, select the well to use for optimization. Any assay well containing diluent and sample is sufficient.

1	1	2	ng over: \ 3	5	6	1	7	1	8	1	9	10	1	11	1	12
1																
E																
-																
G																

Click next, and optimization will proceed. A progress indicator will appear. Once optimization is complete, you can save the optimized read height; you also have the option to customize the read height.

Optimizati Verify the Optimized Rea field. Click Save to save t	d Height. You can a	DICIC adjust the height, if desired, by editin	ig the value in the Cust	om Read Height
Optimized Read Height	3.50 mm	Custom Read Height 3.50	mm 🗧	

Once both optimizations are complete, click "Read Plate" to read using your optimized microplate definition and optimized read height.

Pre-Read Optimization Opt	ions ×
Optimization Options	•
	ation before reading the plate. To improve acy, run Microplate Optimization each time ges.
the measurement accura	ment before reading the plate. To improve scy, run Read Height Adjustment each time rrent read height: 3.50 mm above the Plate.
	Run Optimization
Microplate Options	
	Plate is Lidded
	Read Plate Cancel

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