Configuring the SpectraMax® M5, M5e, and FlexStation® 3 Multi-Mode Microplate Readers for ValitaTiter Assays

Setup Guide

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MOLECULAR DEVICES

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The SpectraMax M5, M5e, and FlexStation 3 Multi-Mode Microplate Readers have been validated for use with the ValitaTiter and ValitaTiter Plus assays. Optimal instrument settings are the same for all three readers.

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 M5, M5e, or FlexStation 3 reader are shown below. Be sure to insert the purple (SpectraMax M5 or M5e reader) or black (FlexStation 3 reader) plate adapter in the plate carriage prior to reading the assay plate.

ValitaTiter

Parameter	Setting
Read Mode	FP
Read Type	Endpoint
Excitation	485 nm
Emission	525 nm, 515 nm cutoff
Plate Type	96-well: 96 Well Corning Half Area
PMT gain	Medium
Flashes per read	100
Settling time (ms)	100
G factor (set in Data Reduction)	1.0

ValitaTiter Plus

Parameter	Setting
Read Mode	FP
Read Type	Endpoint
Excitation	485 nm
Emission	525 nm, 515 nm cutoff
Plate Type	96-well: 96 Well Corning Half Area
PMT gain	Low
Flashes per read	100
Settling time (ms)	100
G factor (set in Data Reduction)	1.0

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 M5, M5e, or FlexStation 3 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. #I2511)
- 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 µg/mL for ValitaTiter, or 2000 µg/mL for ValitaTiter Plus) and including a standard with no IgG (standard 8, 0 µg/mL).

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

- ValitaTiter: Make up 600 µL of standard 1 at 100 µg/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 µL 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 M5, M5e, or FlexStation 3 reader using the optimized settings shown above.

Contact Us

Phone: +1.800.635.5577
Web: www.moleculardevices.com
Email: info@moldev.com
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Regional Offices

USA and Canada	+1.800.635.5577	Taiwan/Hong Kong	+886.2.2656.7585
United Kingdom	+44.118.944.8000	Japan	+81.3.6362.9109
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China	+86.4008203586	India	+91.73.8661.1198

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