Omnia® Assay Setup Guide on the Tecan Infinite® M1000 Microplate Reader

NOTE: The Tecan Infinite® M1000 Microplate Reader was tested for compatibility with Invitrogen's Omnia® Assay using the Omnia® Y Peptide 12 kit (KPZ3121) and JAK2 JH1/JH2 and JAK2 JH1/JH2 V617F kinases. The following document is intended to demonstrate setup of this instrument. For more detailed information and technical support of Invitrogen assays please call 1-800-955-6288, select option "3", then extension 40266. For more detailed information and technical support of Tecan instruments or software, please contact Tecan at 1-888-798-0538 or info@tecan.com.

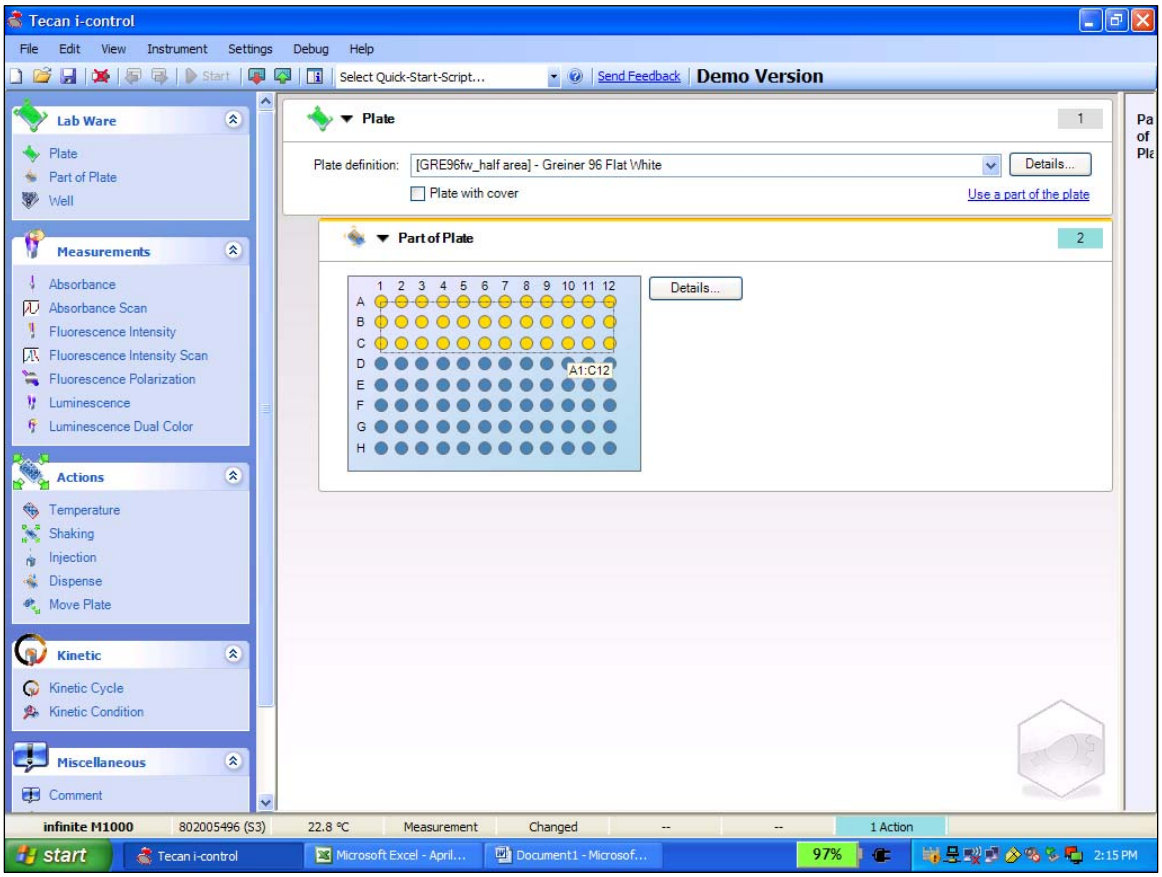
A. Recommended Optics

	wavelength (nm)	diameter (mm)
Excitation	360/12	monochromator
Emission 1	485/12	monochromator

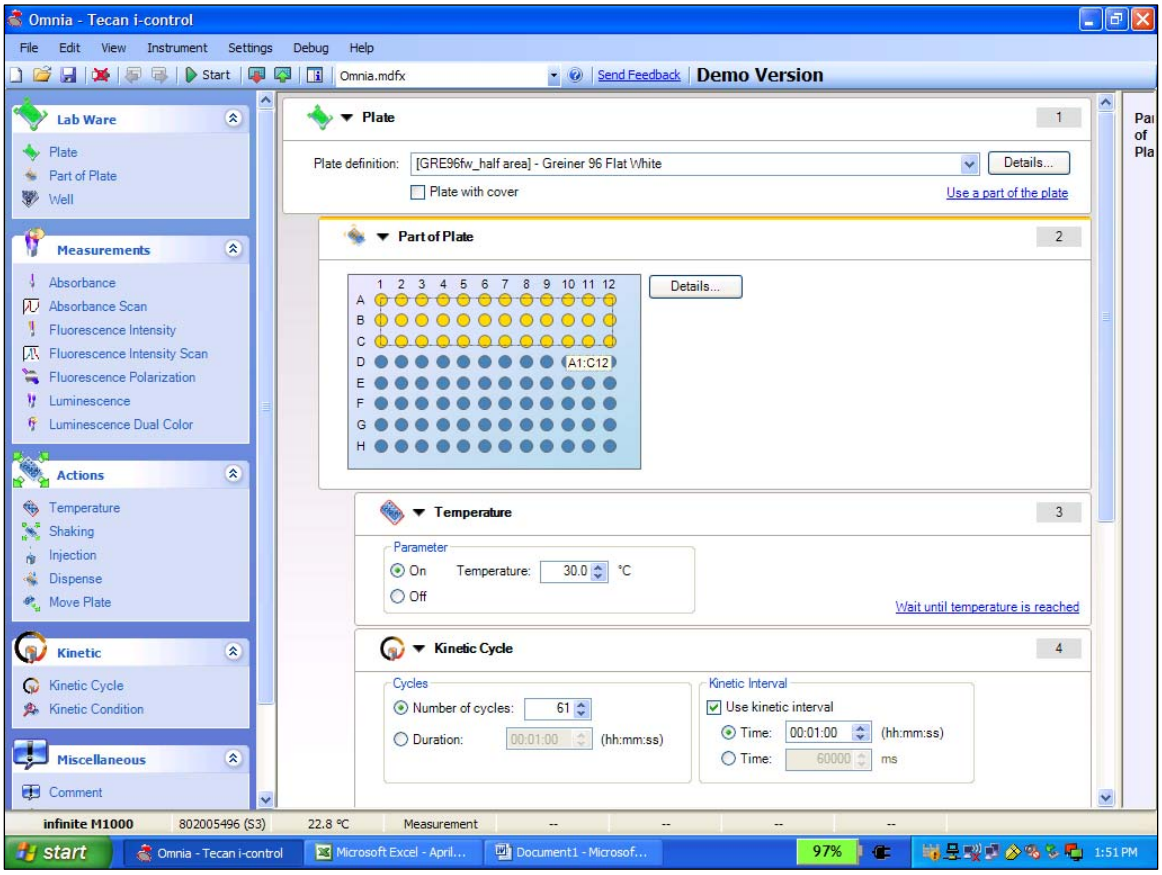
B. Instrument Setup

1. Make certain plate reader is turned on, and open up Tecan i-Control software on computer.

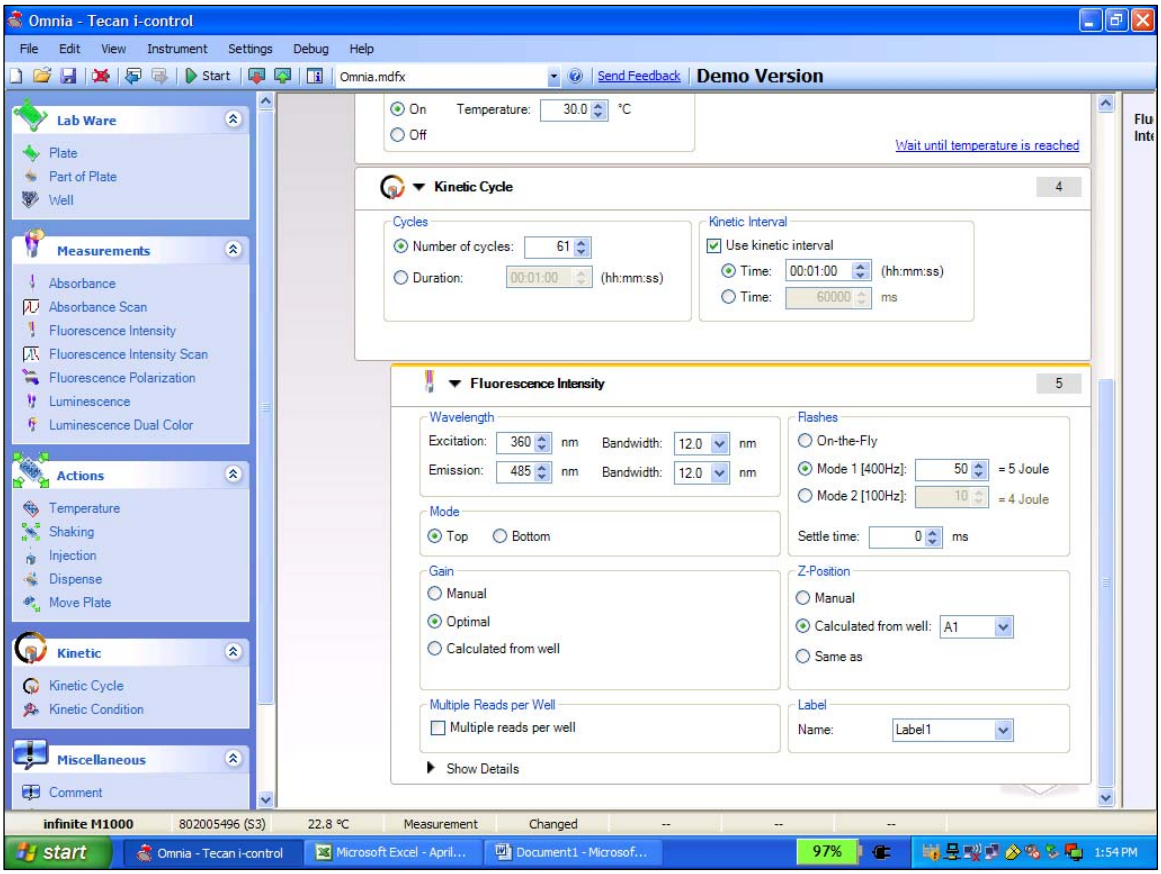
2. When i-Control opens, it will default to a generic starting page. Select "Plate Out" from the menu at the top to open the carriage, and insert your plate, then select "Plate In" to load your plate into the reader. Select your plate definition from the drop-down menu. (Note in this case we have selected 96-well white half-volume plates, as all assays were performed in 40 µl volumes.) Next, from the "Measurements" tab at the left side of the screen, select "Fluorescence Intensity".



3. At this point, a settings tab will open (below). Select the portion of the plate you wish to read by dragging across with your mouse to highlight selected wells. In the "Actions" tab on the left, select "Temperature" and set the temperature for 30 degrees celsius. Next, from the "Kinetics" tab on the left, select "Kinetic Cycle" and enter 61 cycles and 1:00 minute kinetic intervals as shown below.



4. After setting up the temperature and kinetic parameters, select "Fluorescence Intensity" from the "Measurements" tab. Set the appropriate Excitation and Emission values as shown below, as well as Flashes, Z-Position, and Gain (be sure to set the Z-Position to a well containing fluorescent probe).



5. Once all settings have been selected, and a plate is inserted and ready to read, select "Start" from the top menu bar to read.

C. Omnia® Kinase Assay using JAK2 JH1/JH2 and JAK2 JH1/JH2 V617F

NOTE: The following is a sample titration assay performed for demonstration purposes. This section is only an explanation of the procedure followed in generating the data for Section D and not recommended as a control protocol. We recommend all first-time users follow the provided protocols and/or validation packets specific for their assay, and run proper controls. The instrument settings above would be sufficient for any Omnia® kinase assay, the information below is provided as representative data. Assay was run in 50 µl using 1 µM kinase inhibitor and at a kinase concentration of 435 ng kinase per well, based upon recommendations from R&D. Concentration of kinase used will vary, for more information please see the Omnia® kinase assay protocol for your specific kinase of interest at the following link: http://www.invitrogen.com/content.cfm?pageid=11338&cid=fl-OMNIA.

1. Prepare initial inhibitor solutions at 10X from stock, in deionized water. Add 5 µl 10 µM inhibitor to proper wells. Note in the plate outline shown below, 5 µl Staurosporine added to wells A1-A3 and C1-C3 and 5 µl JAK2 Inhibitor II added to wells A4-A6 and C4-C6. Add 5 µl water alone to wells B1-B6, B10-B12, and D1-D3 (see Figure 1).

		1	2	3	4	5	6	7	8	9	10	11	12
JH1/JH2	A	**Staurosporine**			**JAK2 In II**			**No inhib.**			**no kinase**		
	B										**100% Phos**		
V617F	C	**Staurosporine**			**JAK2 In II**			**No inhib.**			**no kinase**		
	D												
	E												
	F												
	G												
	H												

Figure 1: Schematic of Omnia® plate layout. Staurosporine and JAK2 Inhibitor II were assayed at 1 µM in triplicate against both JAK2 JH1/JH2 and JAK2 JH1/JH2 V617F. Note no inhibitor controls were also run for both kinases to determine full kinase activity, as well as a kinase-free control and a phosphopeptide control.

2. Master Mix minus inhibitor prepared as follows (quantities are per well, in this case multiplied by 26 to allow for 24 reactions plus excess):

Kinase Reaction Buffer (10X)	5 µl
Tyrosine Peptide Substr. (dilute to 10X)	5 µl
ATP Solution (dilute to 10X)	5 µl
DTT Solution (dilute to 10X)	5 µl
Deionized Water	20 µl

3. Add 40 µl Master Mix per well to wells A1-A6, B1-B6, C1-6, and D1-D3. Add 5 µl Omnia® Tyr Phosphopeptide Control and 5 µl all other components above except substrate to wells B10-B12, and deionized water to a total of 50 µl.

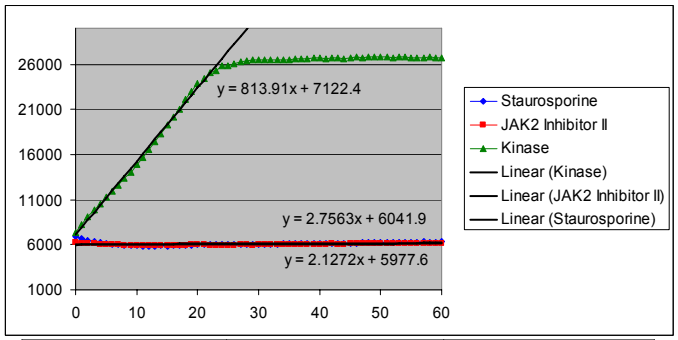
4. Allow plate to pre-incubate 5 minutes at 30º C.

5. Add 5 µL JAK2 JH1/JH2 to wells A1-A12 and B1-B3, and 5 µl JAK2 JH1/JH2 V617F to wells C1-C12 and D1-D3 (both kinases pre-diluted to 85 µg/ml).

6. Read and analyze as directed in the protocol.

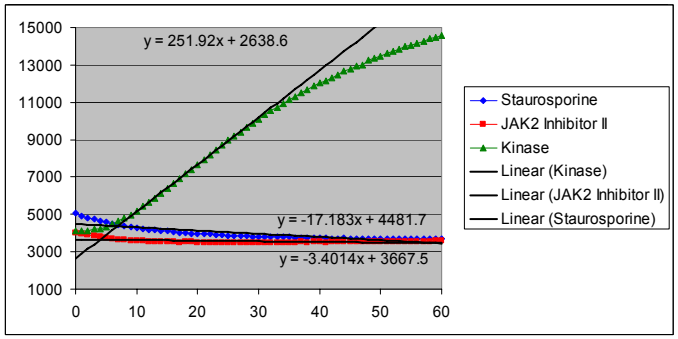
D. Results:

JAK2 JH1/JH2



Rxn Cond.	Rate (RFU/min)	% Inhibition
Kinase	813.91	0
Staurosporine	2.76	99.70
JAK2 Inhibitor II	2.13	99.74

JAK2 JH1/JH2 V617F



Rxn Cond.	Rate (RFU/min)	% Inhibition
Kinase	251.92	0
Staurosporine	-17.18	106.80
JAK2 Inhibitor II	-3.40	101.35

Figure 2: Omnia® Kinase Assay. Omnia® assay performed as described above and read on the Tecan Infinite® M1000. Assay results monitored at 1-minute intervals, data graphed in Microsoft Excel.