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**Setup Guide on the Tecan Infinite® M200 Microplate Reader**

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## **Z'-LYTE® Assay Setup Guide on the Tecan Infinite® M200 Microplate Reader**

The Tecan Infinite® M200 Microplate Reader was tested for compatibility with Invitrogen's Z'-LYTE® Assay using the Z'-LYTE® Tyr6 kit (PV4122) against MET kinase (PV3143). The following document is intended to demonstrate setup of this instrument. F200-series instruments would be set up in the same fashion, with appropriate filters. For more detailed information and technical support of Invitrogen assays please call 1-800-955-6288, 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](mailto:info@tecan.com).

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For more detailed information and technical support of Life Technologies assays including specific conditions for assay windows between 2-3 fold, please call 1-800-955-6288 and enter extension 40266 or email [drugdiscoverytech@lifetech.com](mailto:drugdiscoverytech@lifetech.com).

For more detailed information and technical support of BioTek instruments or Gen5 software, please call 1-888-451-5171.

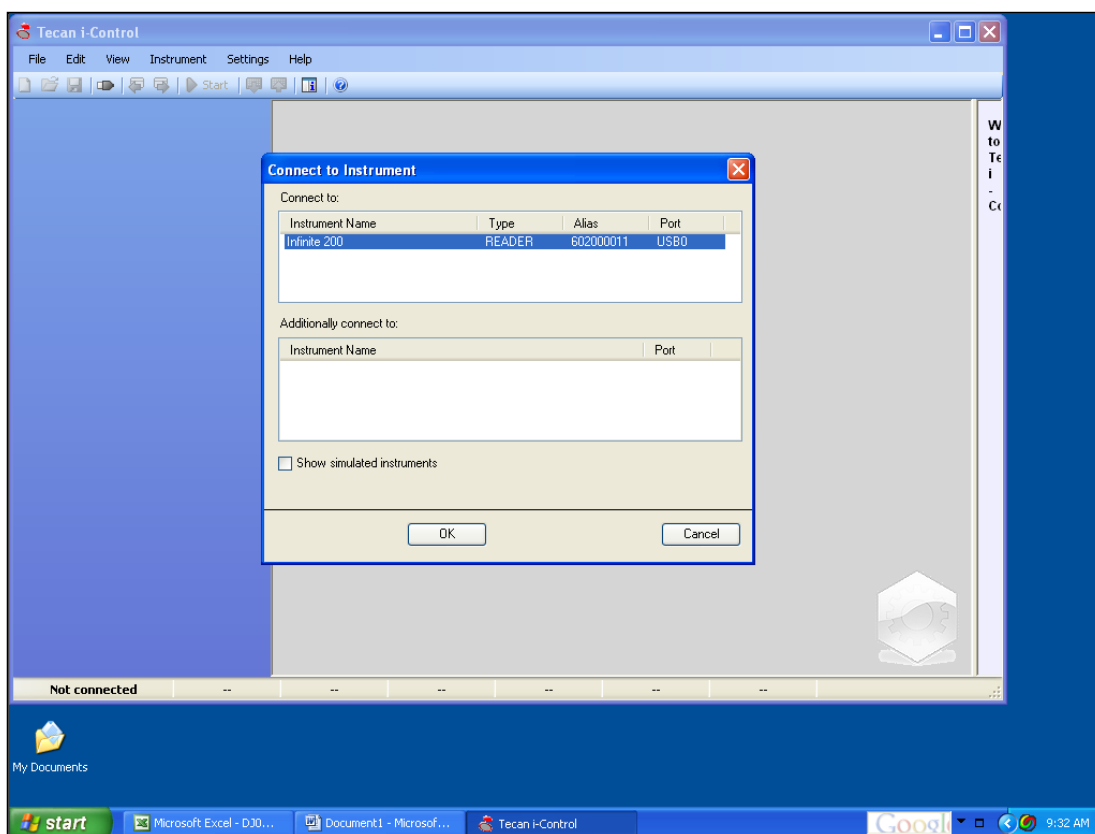
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**A. Recommended Optics**

Tecan Part Number	Wavelength (nm)	Diameter (mm)
Excitation Filter	400/9	Monochromator
Emission Filter 1	445/20	Monochromator
Emission Filter 2	519/20	Monochromator
Dichroic Mirror		

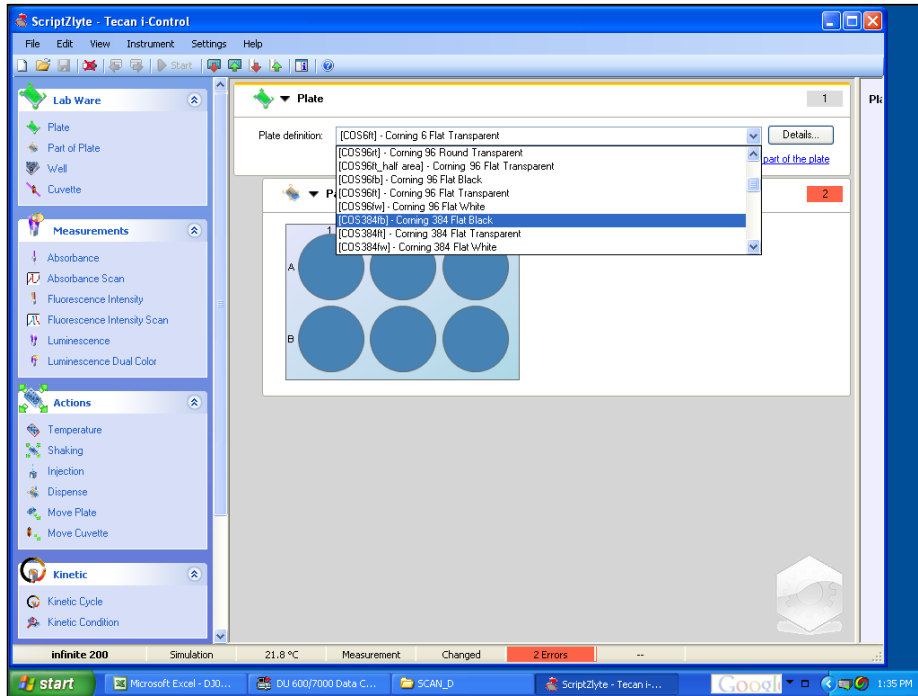
**B. Infinite® M200 Setup**

1. Make certain Infinite plate read is turned on, and open up Tecan i-Control software on computer.
2. “Connect to Instrument” window will automatically pop up to enable the software and instrument to communicate. The following screen appears. Select your instrument and click “OK”.

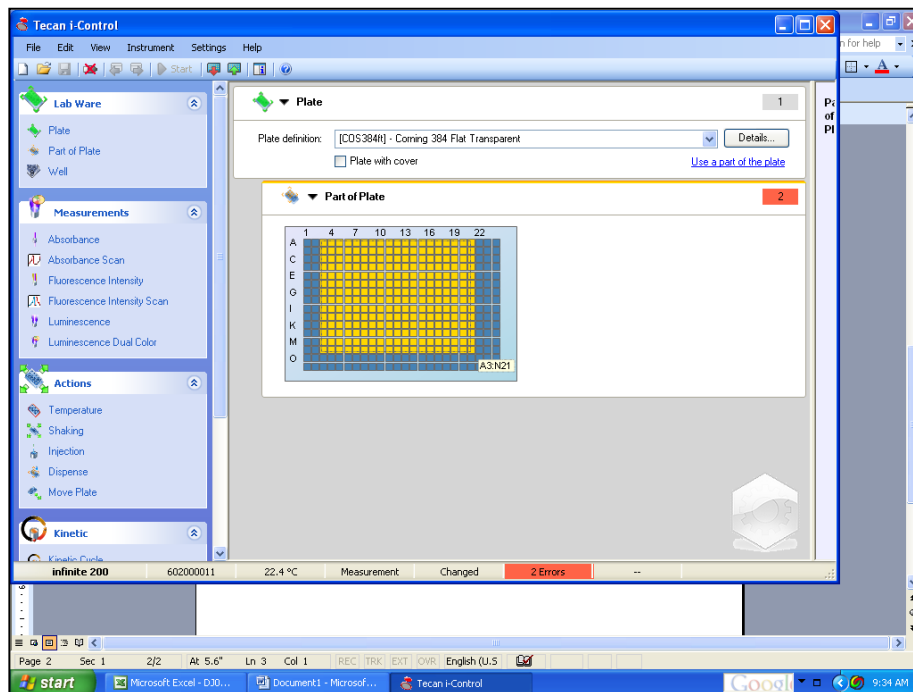


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- In the i-Control menu, choose the appropriate plate definition from the list of available plates. If your plate is not listed, you may have to select an “equivalent” plate or add your plate definitions to the i-Control software.

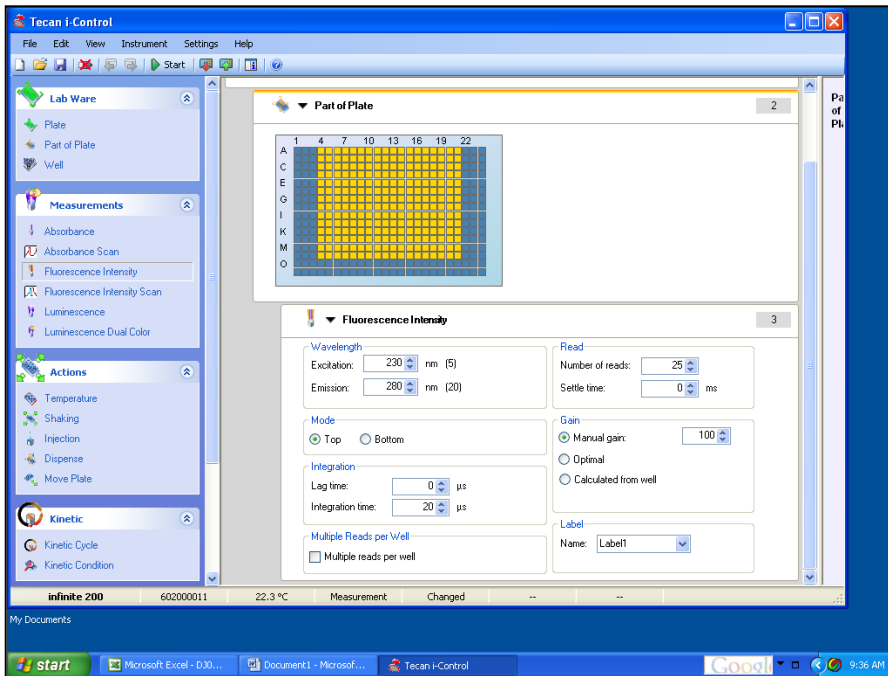


- Select the wells you wish to read by clicking on the first well and dragging to highlight the wells to be read.

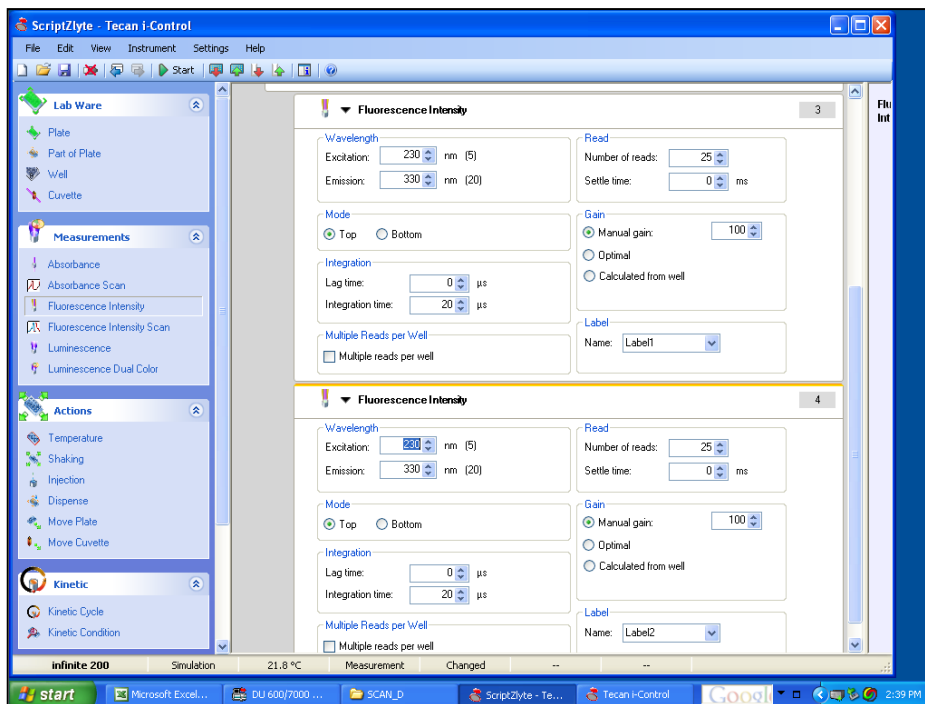


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- For Measurement 1, click on “Fluorescence Intensity”. This opens an instrument settings window below the plate selection tool:



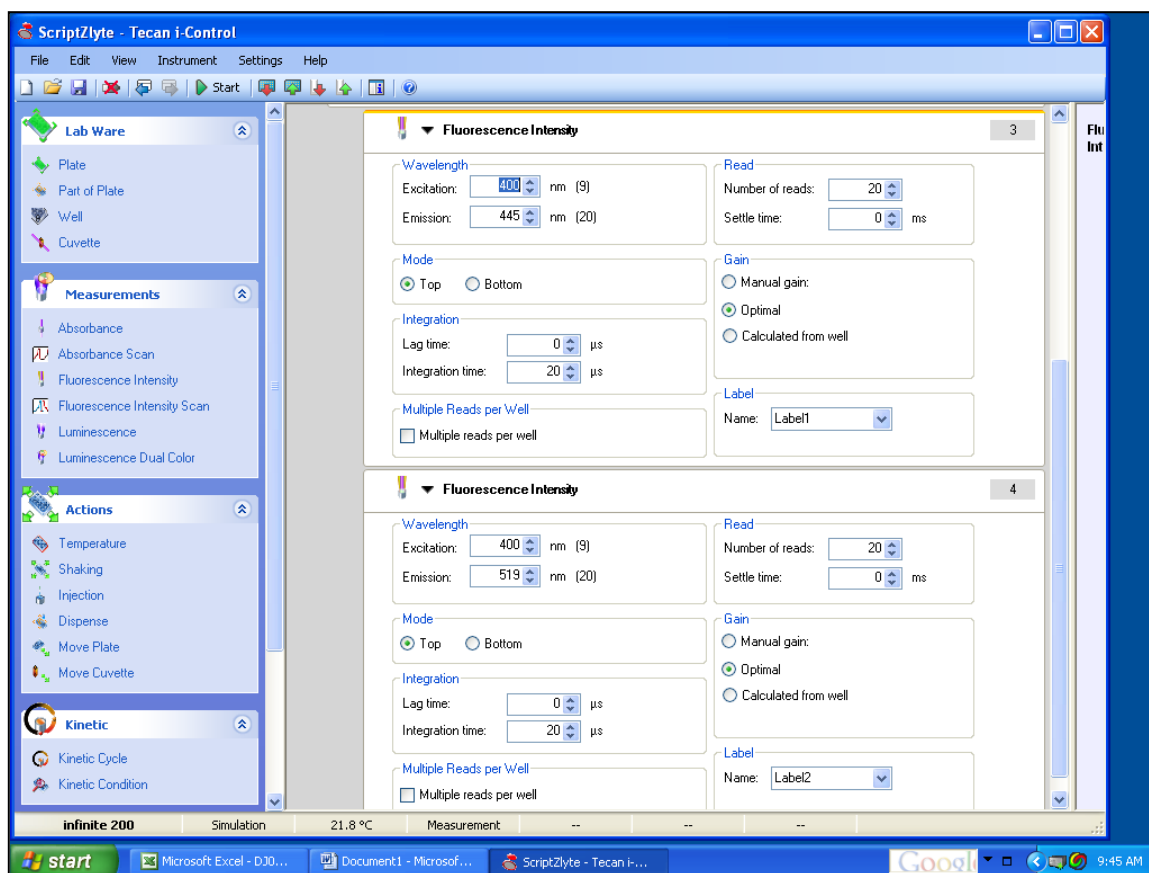
- Z'-LYTE<sup>®</sup> is a FRET-Based assay technology, requiring measurement of both a donor and an acceptor. Clicking the “Fluorescence Intensity” icon again will open a second instrument settings window for FRET measurement.



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- For our validation experiments, we used the settings outlined below. Use the drop-down tabs and/or typing manually to set the excitation, emission, number of reads, select the method of setting the gain, etc.

**NOTE:** The Infinite® M200 series instrument has a defined bandwidth feature, so the monochromator will automatically set bandwidth. Also, we used 20 reads per well and optimal gain in this experiment. Reducing the number of reads per well to 5 will reduce the read time per plate, and gain can also be calculated from defined wells or manually.



- Click on the “Move Plate Out” icon in the top toolbar to open the Infinite. Place your plate in the instrument and click on the “Move Plate In” icon to close the instrument. Click on the “Start” icon on the top toolbar, and the instrument will begin reading. An Excel spreadsheet is opened and data will appear as it is generated. Save the worksheet after the machine is done reading. When closing i-Control, you may save the script as well as the prompt in order to keep your instrument settings.

**Have a question?**

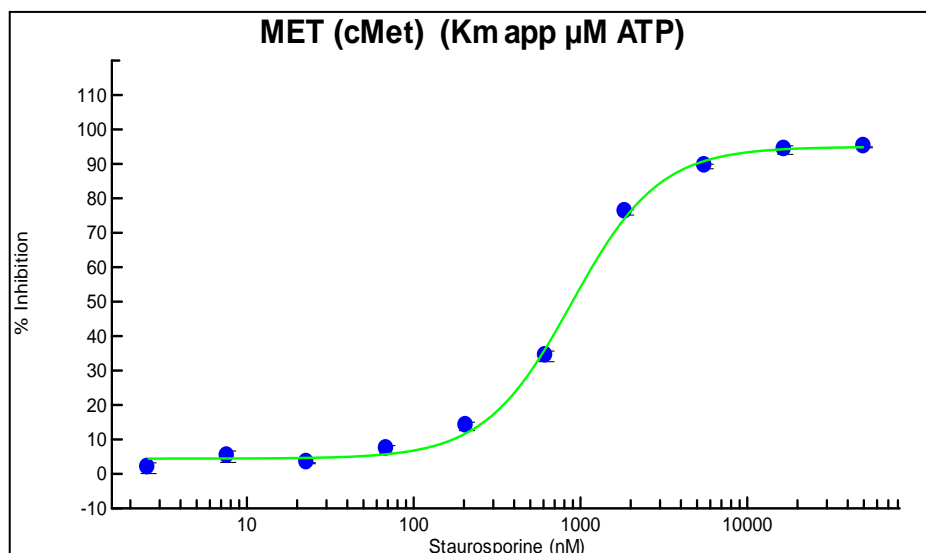
Contact our Technical Support Team

NA: 800-955-6288 or INTL: 760-603-7200 ext. 40266

Email: [drugdiscoverytech@lifetech.com](mailto:drugdiscoverytech@lifetech.com)

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C. Results



**Figure 1: Z'-LYTE® Kinase Assay.** Staurosporine Dose-Response Curve on the Tecan Infinite® M200. Assay was run as directed in Z'-LYTE protocol, at the MET kinase ATP  $K_{m,app}$ .

Instrument	Tecan M200	Tecan Safire™
<b>B/G ratio, 0% phosphorylation</b>	2.368	6.718
<b>B/G Ratio, 100% phosphorylation</b>	0.401	1.379
<b>B/G Ratio, 0% inhibition</b>	1.393	4.371
<b>Fold-Change</b>	5.8	4.9
<b>Z'</b>	0.85	0.88

**Table 1: Result Comparison.** Blue/Green FRET ratios, assay windows (Fold-change), and Z' values are compared for the same experiment read on the Tecan Infinite® M200 and Tecan Safire™ 2.