Adapta® Assay Setup Guide on the Tecan Safire²™ Microplate Reader

NOTE: The Tecan Safire²™ Microplate Reader was tested for compatibility with Invitrogen's Adapta® Europium-based TR-FRET Assay using the Adapta® Universal Kinase Assay Kit (PV5099) and poly E4Y substrate against 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.

NOTE: This setup guide is also compatible for use with Invitrogen's LanthaScreen® Europium-based TR-FRET Assays (e.g. LanthaScreen® Eu Kinase Binding Assay).

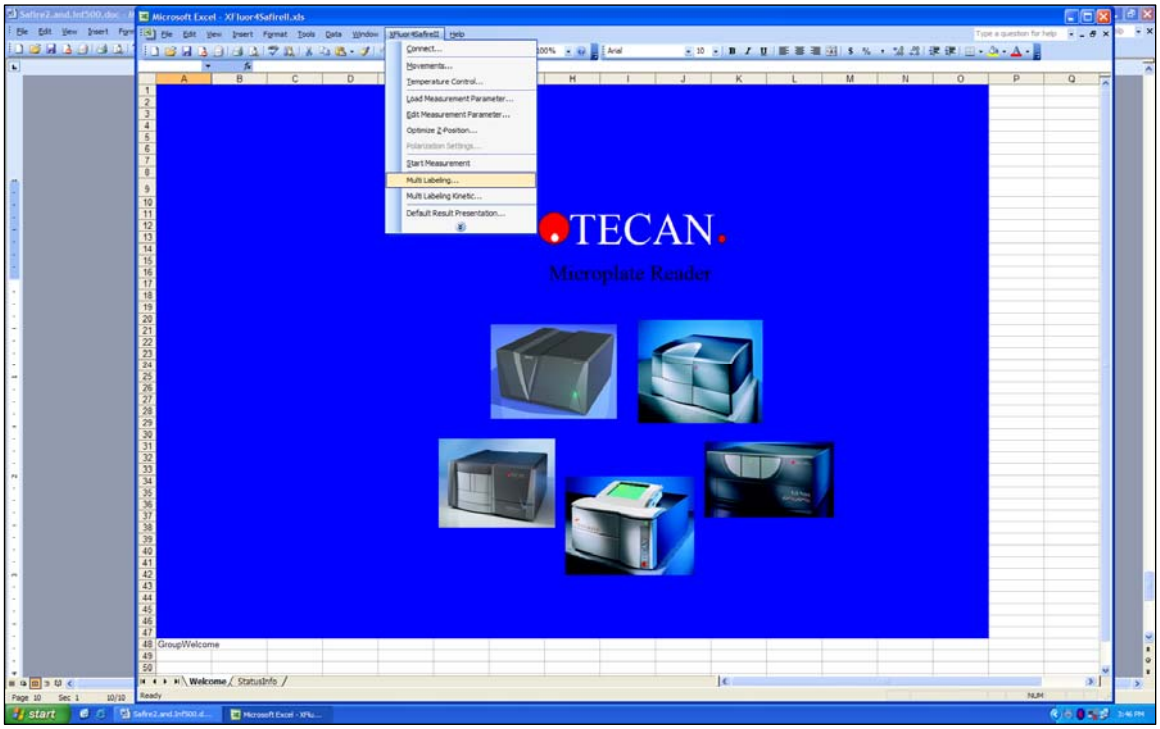
A. Recommended Optics

	wavelength (nm)	diameter (mm)
Excitation	317/20	monochromator
Emission 1	620/12	monochromator
Emission 2	665/12	monochromator

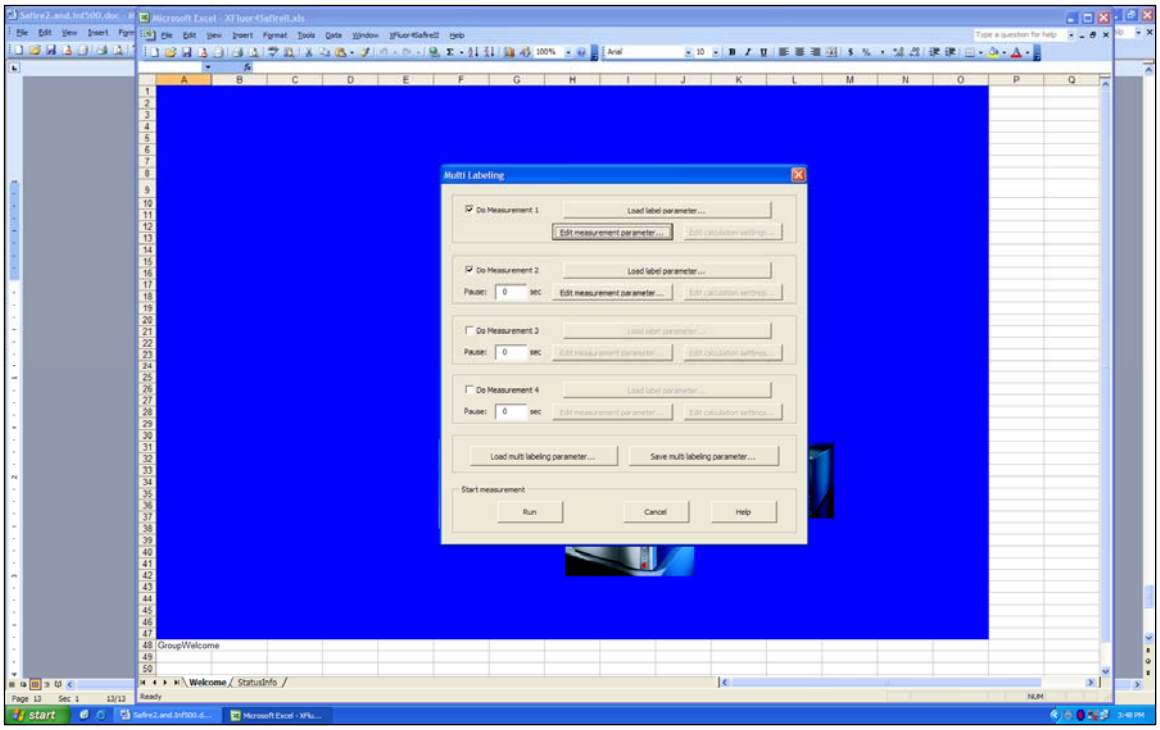
B. Instrument Setup

1. Make certain plate reader is turned on, and open up XFluor Data Manager software on computer.

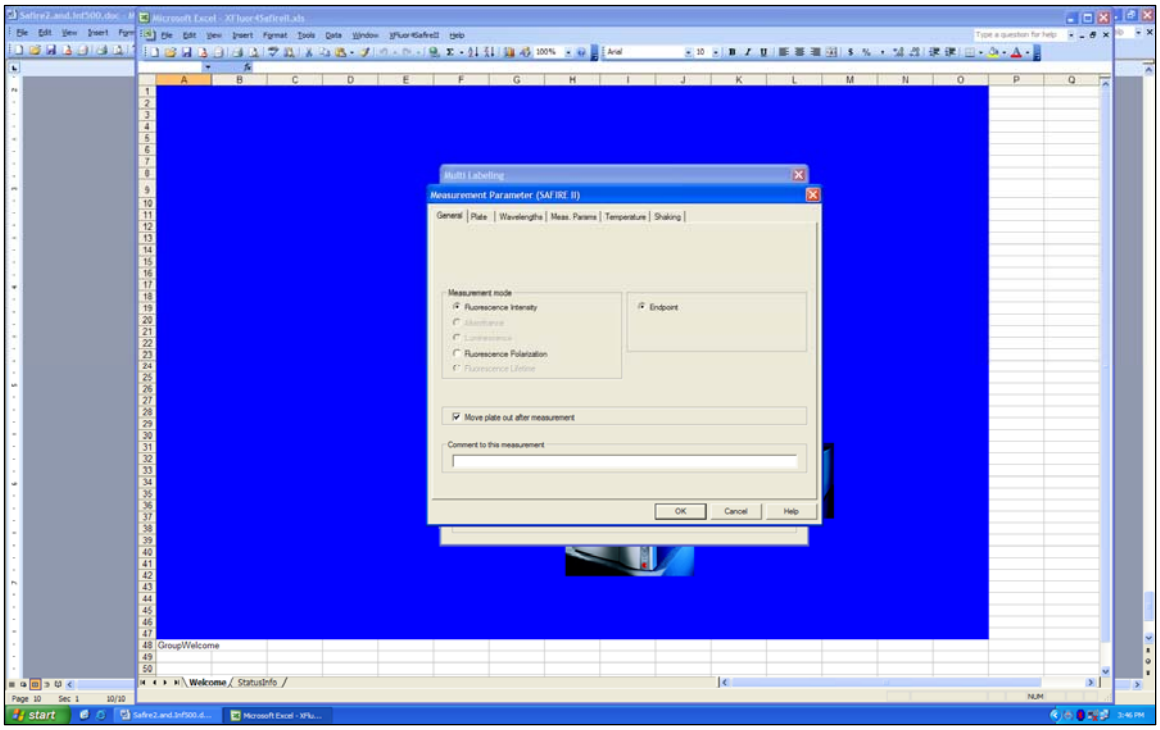
2. When XFluor opens, select "Multi-Labeling" from drop-down menu bar at the top of the screen.



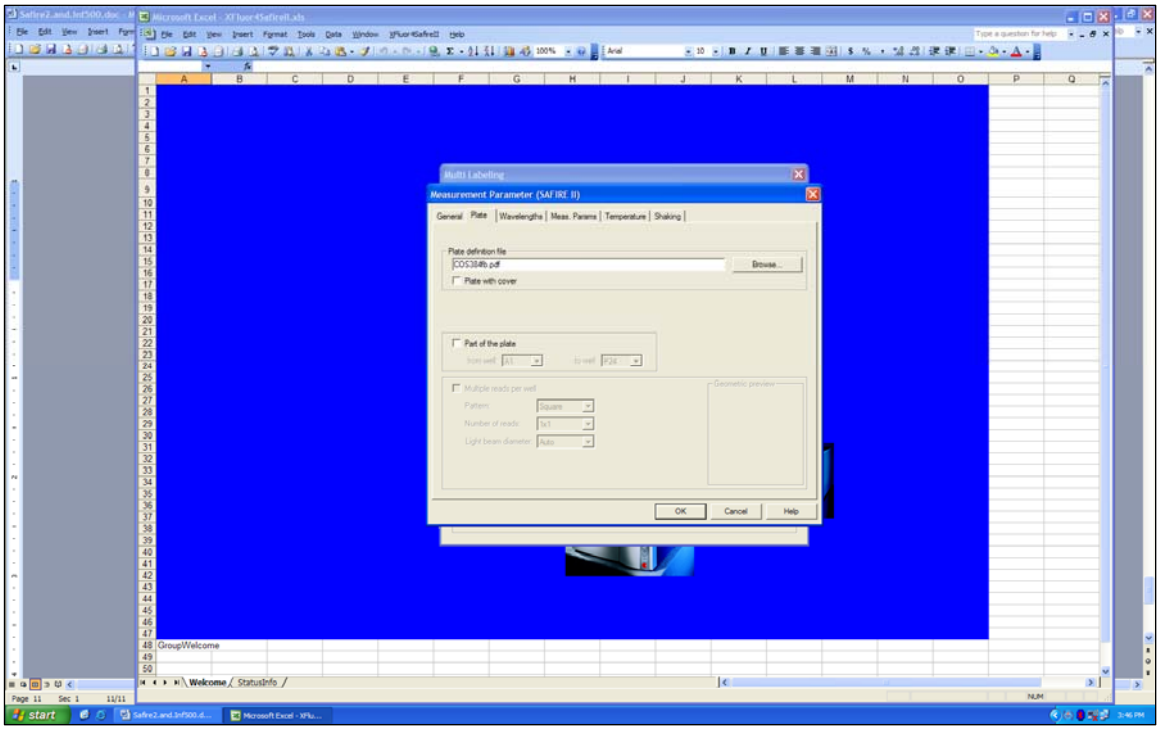
3. A Multi Labeling window will appear. Check the "Do Measurement 1" box, and then click on the "Edit Measurement Parameter" tab.



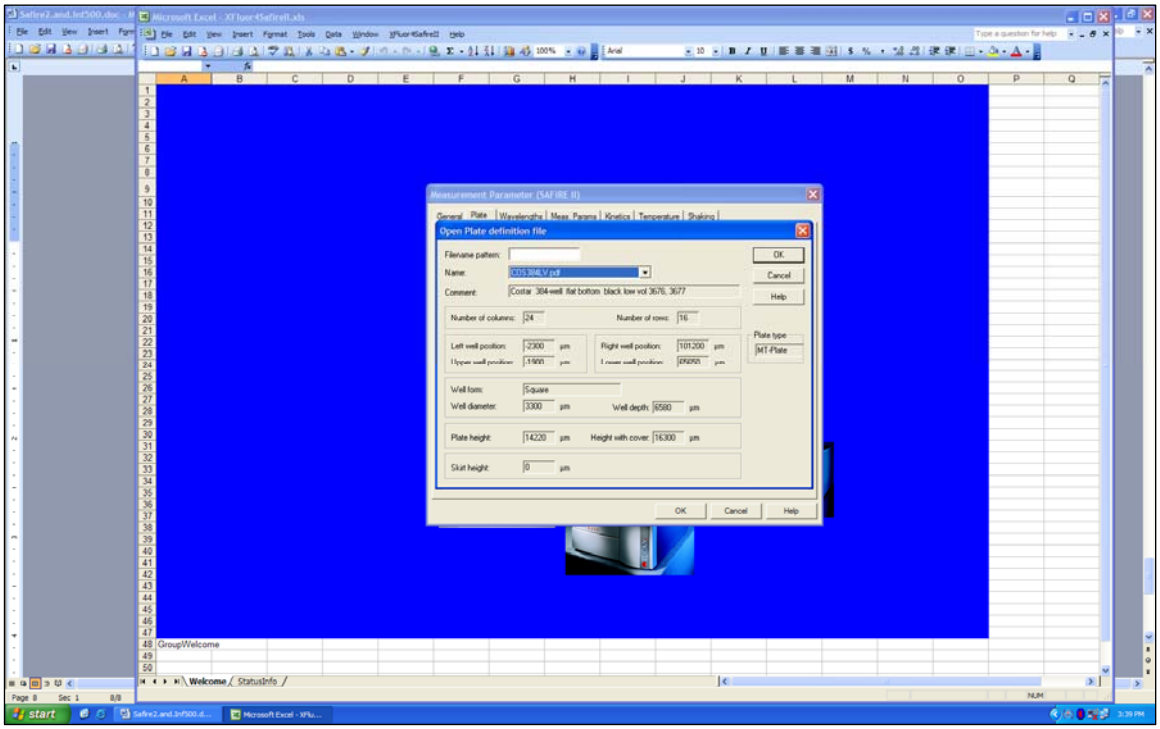
4. A popup Measurement Parameter window will appear. Under the "General" tab select the "Fluorescence Intensity" and "Endpoint" buttons.



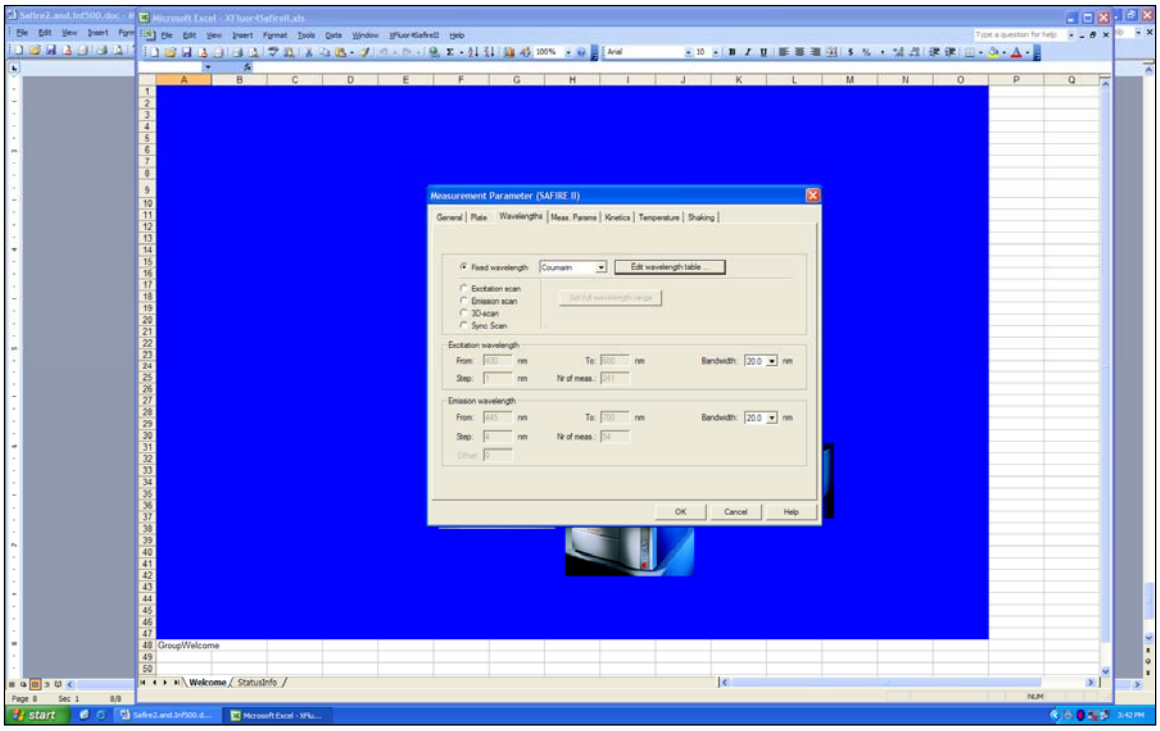
5. Now select the “Plate” tab at the top of the window. When the new tab opens, click on the "Browse" tab. Note this is also where to select which wells to read if only reading part of the plate.



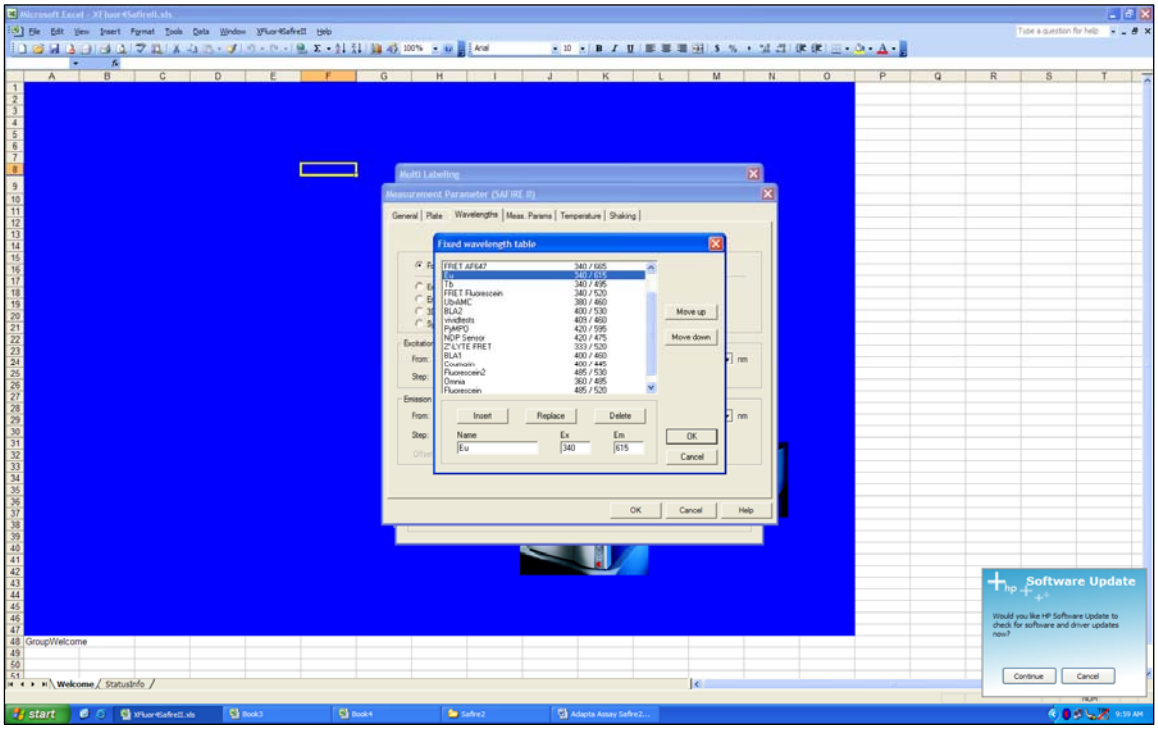
6. When the new plate window appears, select your plate as shown below. Note we have run Adapta® using both white and black plates on this reader and obtained similar results. Click OK when finished to return to the main protocol setup window.



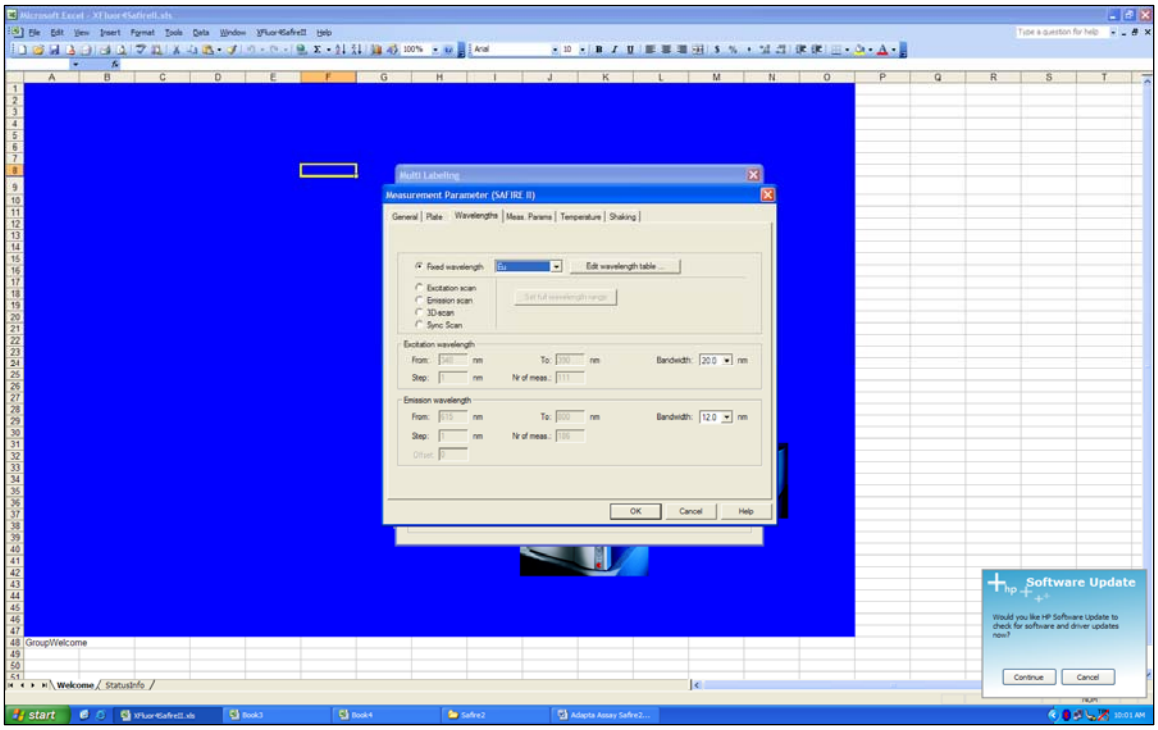
7. Select the "Wavelengths" tab from the top. Next, click the "Edit Wavelength Table" button.



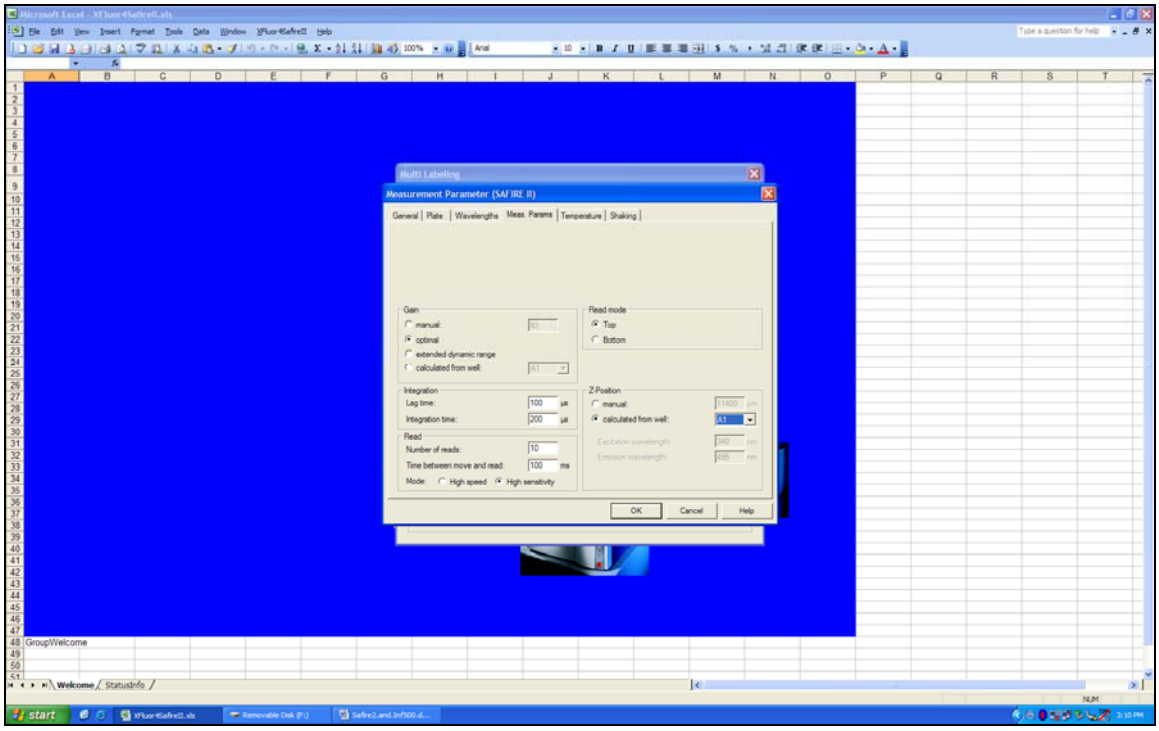
8. A new popup will appear. If you do not have suitable settings already loaded, enter your excitation and donor emission wavelengths in the blanks below "Name", "Ex", and "Em", respectively. When you have entered the desired settings, select "Insert" to enter then into the table of defined settings as shown, and then select OK when finished.



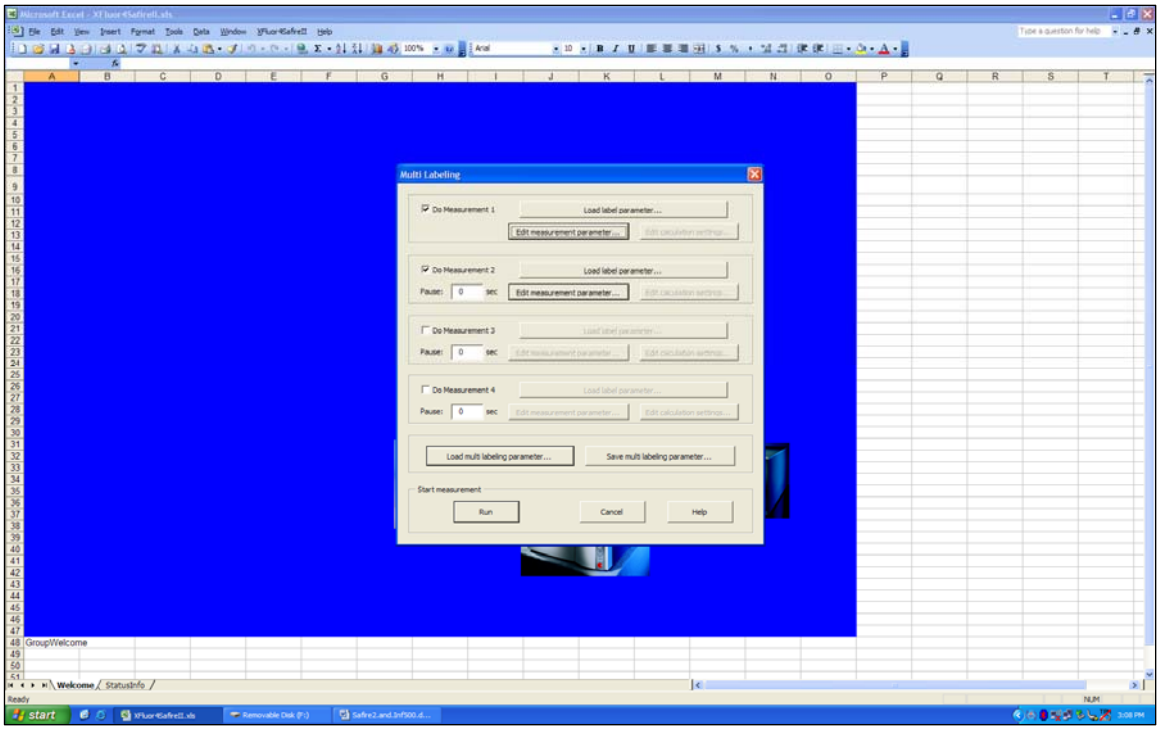
9. When returned to the main protocol window, set your excitation and emission bandwidths as shown below, then select the "Meas. Params" tab.



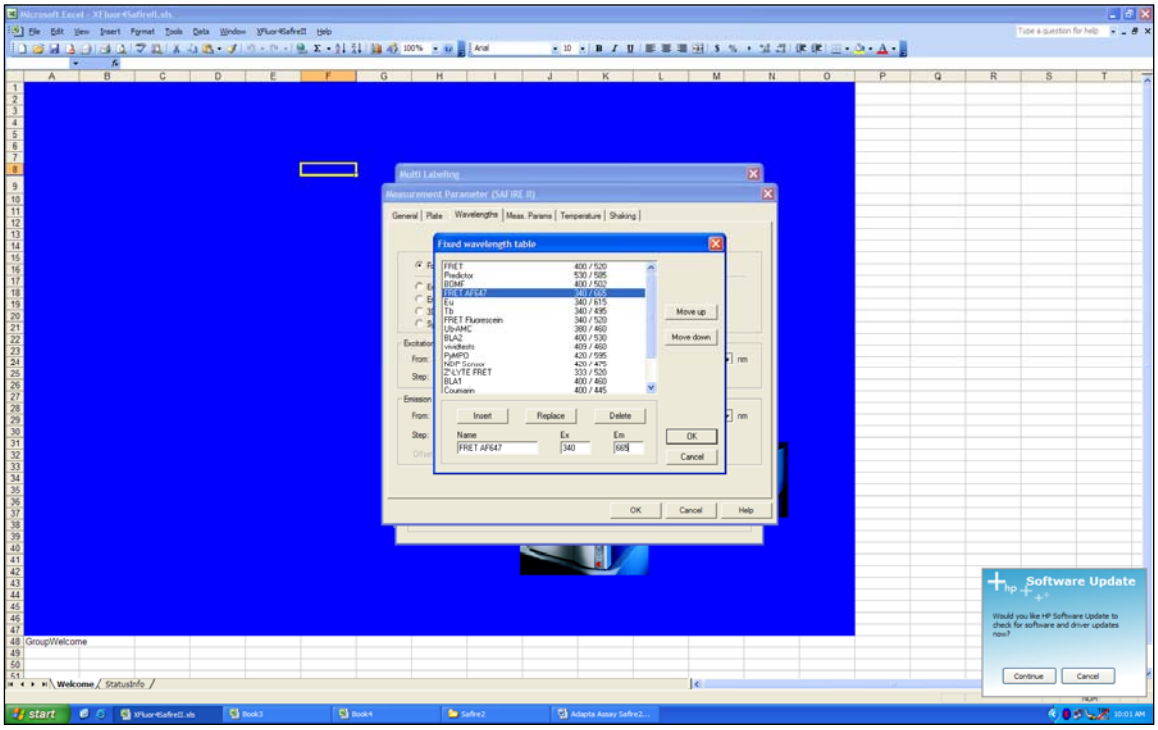
10. Set the Gain for “Optimal”, 10 reads, and set the Read Mode to "Top". Select a suitable well for Z-Position. LanthaScreen is a Time-Resolved FRET assay, so set the Lag and Integration times to 100 and 200 µseconds, respectively. When finished, select OK.



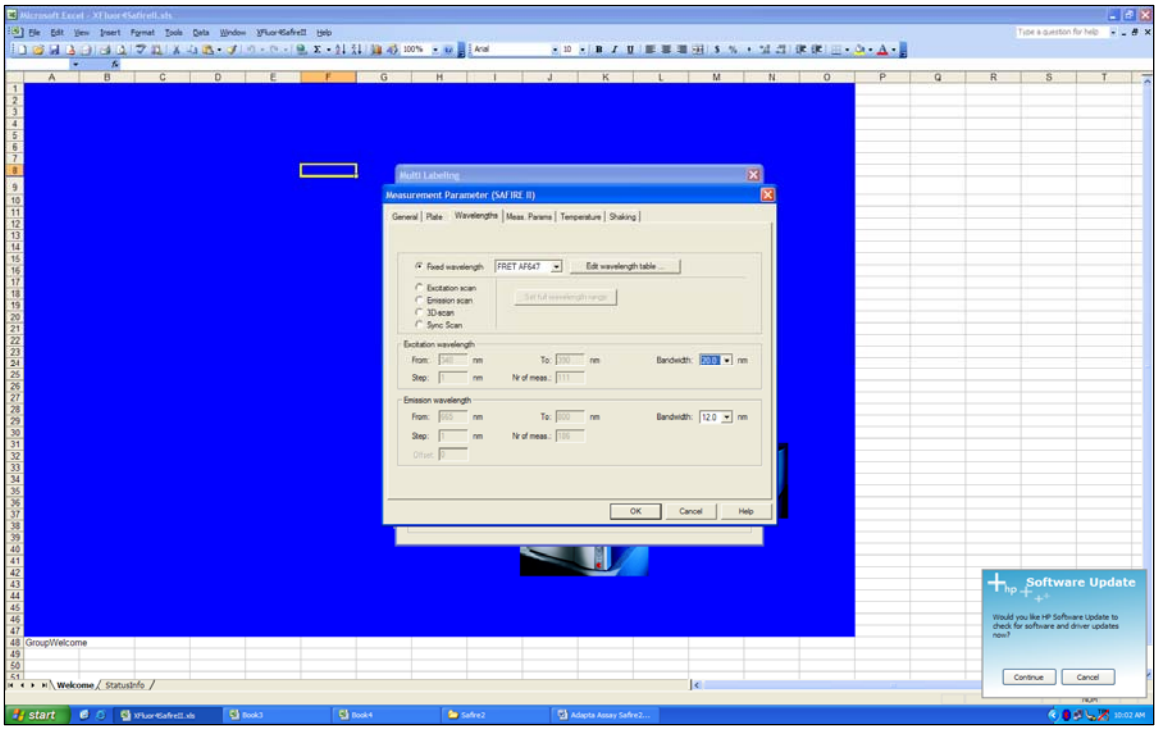
11. You will be returned to the Multi-Labeling window. Check the "Do Measurement 2" box and click the "Edit Measurement Parameter" tab to enter your excitation and acceptor settings.



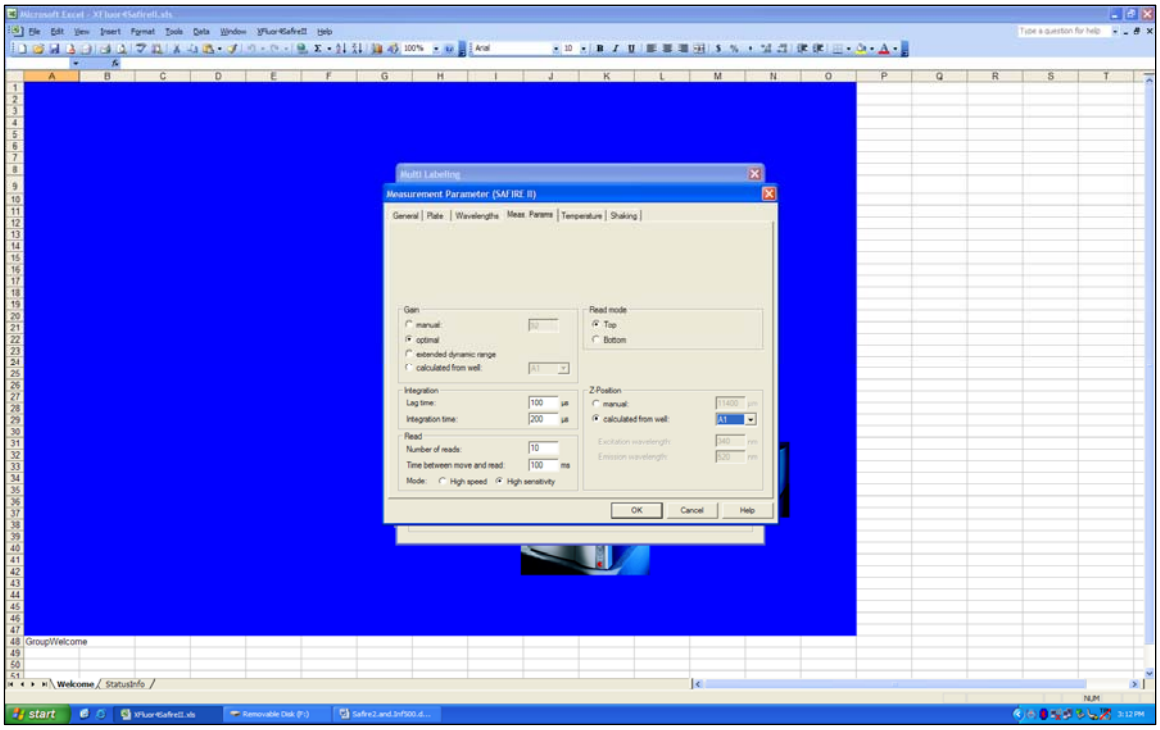
12. Both the "General" and "Plate" tabs will now be set, as their settings were already determined by the first set of measurement parameters entered. Go straight to the "Wavelengths" tab, and select "Edit Wavelength Table" as before. In the popup, enter the values for your excitation and your acceptor emission as shown below, and again select "Insert" to enter them into your tables, then select OK.



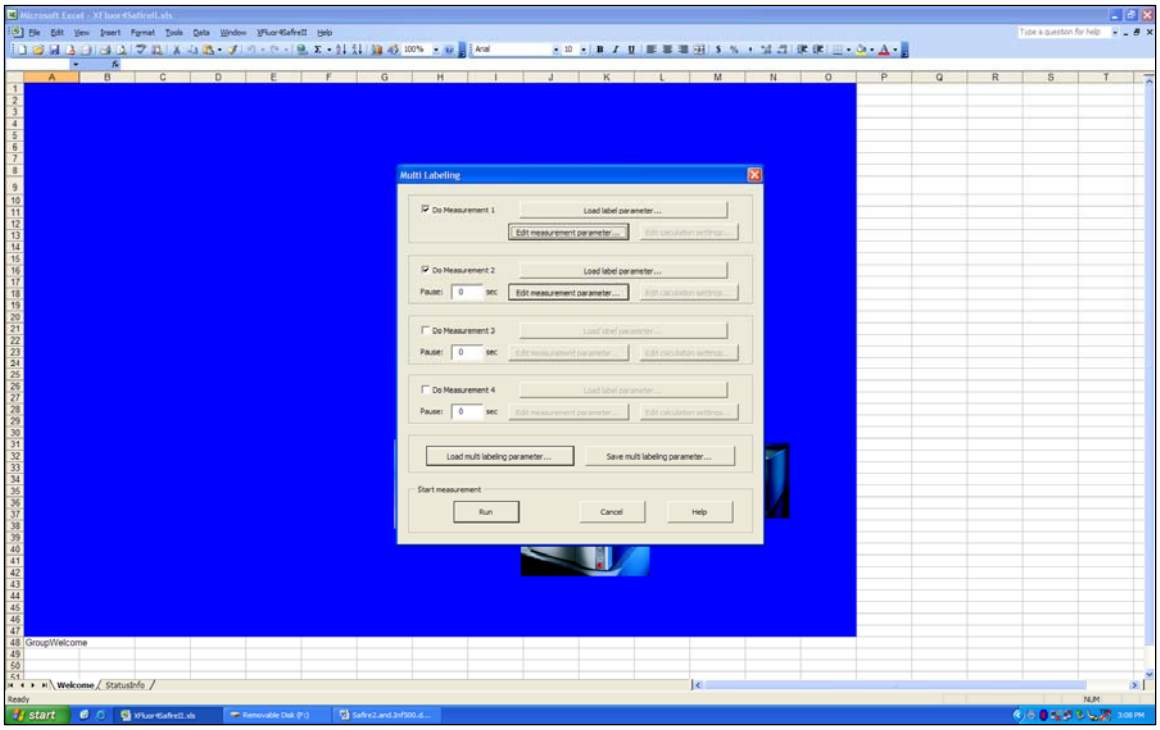
13. You will return to the main protocol page again. Enter the appropriate instrument bandwidths, then select the "Meas. Params" tab.



14. Make sure the Gain is set to "Optimal" and the Read Mode is set to "Top". Select the same well for Z-Position. Set the Lag and Integration times. When finished, select OK.



15. At this point you will default all the way back to the Multi-Labeling window. If you have not already, insert your plate, and select the "Run" tab to read.



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

NOTE: The following is a sample titration assay performed for demonstration purposes. The instrument settings above would be sufficient for any Adapta® assay, the information below is provided as representative data and this section is an explanation of the experiment performed; it is not intended as a substitute for the provided assay-specific assay protocols and/or validation packets. We recommend all first time users follow the protocols included with their assays, and include proper controls. This assay was run at 50 µM ATP, a figure based upon the ATP Kmapparent determined in Invitrogen's Z'-LYTE® kinase assays for these kinases, and the Tracer concentration recommended on the Adapta® Alexa Fluor® 647 ADP Tracer certificate of analysis. Kinases were used at levels producing approximately 70-80% of maximal phosphorylation. ATP and kinase concentrations should be optimized for each kinase by the actual user and titrations/plate layout may be optimized as well. For more information on setting up assays, consult your protocol or contact Technical Support.

1. Prepare initial 100X serial dilution curves in rows A, and E of a 384-well plate (Figure 1): Dilute Staurosporine and JAK2 Inhibitor II to a 100X initial concentration (100uM) in 100% DMSO. Prepare a set of 1:1 serial dilutions from the initial concentration in a 384-well plate, starting with 80 µl in Column 1 and 40 µl DMSO in wells 2-20. Add 40 µl from well 1 to well 2, and then mix well 2, and take 40 µl from well 2 and add to well 3, mix, and so on.

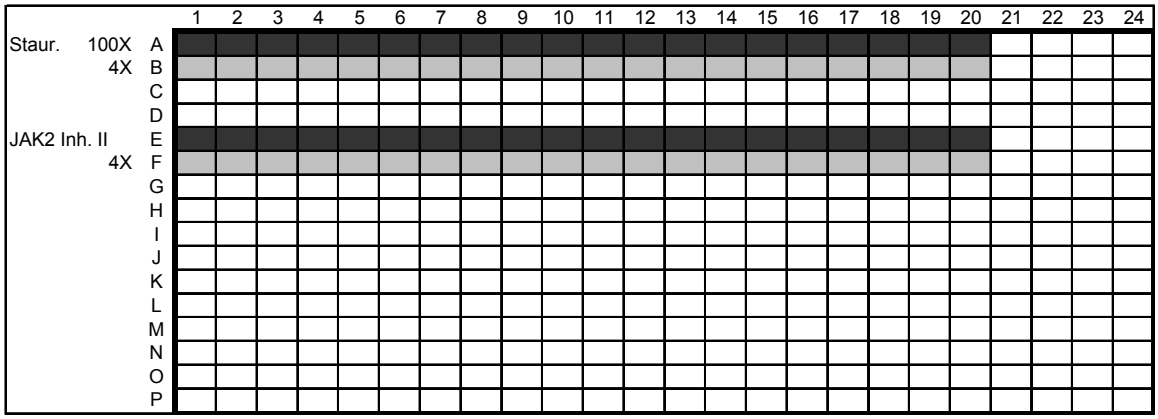


Figure 1: Schematic of initial compound dilution. Staurosporine and JAK2 Inhibitor II were titrated from a 100 µM starting concentration in the initial dilution series by preparing a 1:1 dilution curve in DMSO. A secondary dilution to 4X was then prepared in the rows below the initial dilution curve (lighter gray) using kinase buffer.

2. The 100X serial dilution set is then diluted to a 4X working concentration in Kinase Buffer (PV3189, 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA) in the row below by adding 2 µl of diluted inhibitor from the well above to 48 µl of kinase buffer. This will produce a final serial dilution starting at 4 µM, which will then produce a final assay concentration starting at 1 µM.

3. Begin to prepare an assay plate: Add 2.5 µL of the compound dilutions per well into a low volume, white non-treated 384-well plate (Corning Cat. # 3674), in quadruplicate so rows A-D are staurosporine replicates, E-H are JAK2 Inhibitor 2 replicates, etc.

4. Add 2.5 µl of kinase buffer alone to rows 21 and 22 (0% inhibition no compound control), 23 (0% phosphorylation control, no kinase added) and 24 (Phosphopeptide 100% phosphorylation positive control).

5. Add 2.5 µL of the 4X Kinase Mixture (222 ng/ml JAK2 JH1/JH2 or 270 ng/ml JAK2 JH1/JH2 V617F, determined experimentally as outlined above) to Columns 1-22. DO NOT ADD TO COLUMN 23 OR 24. Add 2.5 µL of buffer alone without kinase to Column 23, rows A-L (0% phosphorylation control) and Column 24, rows A-L (100% phosphorylation control). Add 2.5µl kinase buffer alone to the remaining 8 wells (Columns 23 and 24, rows M-P) as a buffer-only reference.

6. Add 5 µL of 2X substrate/ATP Solution (100 µM ATP and 1000 ng/ml poly E4Y) per well to Columns 1-23 to start reaction. Add 5 µl of 2x substrate/ADP solution (80 µM ATP, 20 µM ADP, and 1000 ng/ml poly E4Y) to Column 24.

7. Shake assay plate on a plate shaker for 30 seconds.

8. Incubate assay plate for 60 minutes at room temperature.

9. Add 5 µl per well of 3X Detection Solution (6nM Eu-Anti-ADP antibody, 30 mM EDTA, and 3X the recommended amount of Tracer diluted in TR-FRET Dilution Buffer) to all wells except wells M-P of Columns 23 and 24 (buffer controls only), instead replace with 10 µl TR-FRET Dilution Buffer supplemented with EDTA only.

10. Shake plate again on a plate shaker for 30 seconds.

11. Incubate for 30 minutes at room temperature.

12. Read and analyze as directed in the protocol.

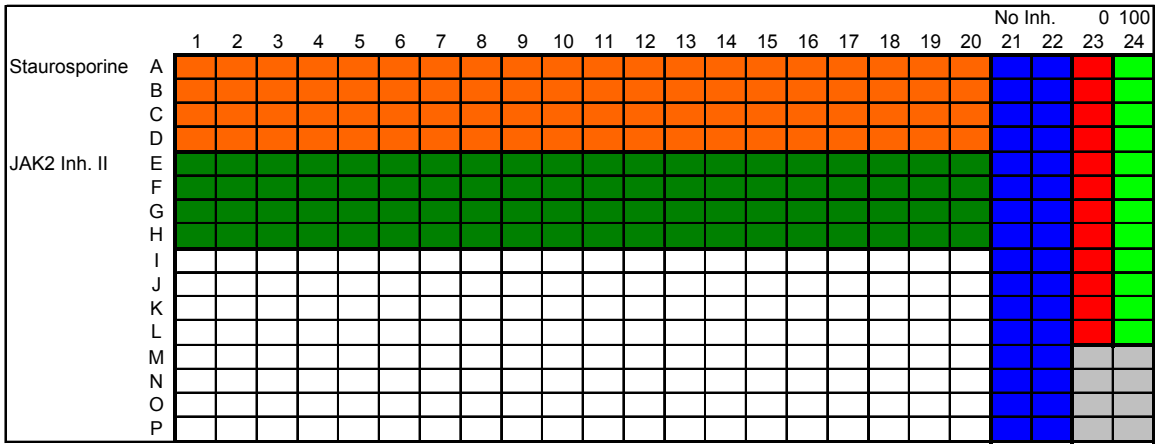


Figure 2: Assay Plate Schematic. Compound titrations shown in Columns 1-20, Columns 21 and 22 prepared without any inhibitor as kinase activity controls, Column 23 prepared with no kinase (0% phosphorylation) and Column 24 prepared using 20% ADP as a positive control (100% phosphorylation). Note 8 wells in gray in bottom right, which were prepared with out any inhibitor, substrates, or antibody as buffer controls.

D. Results:

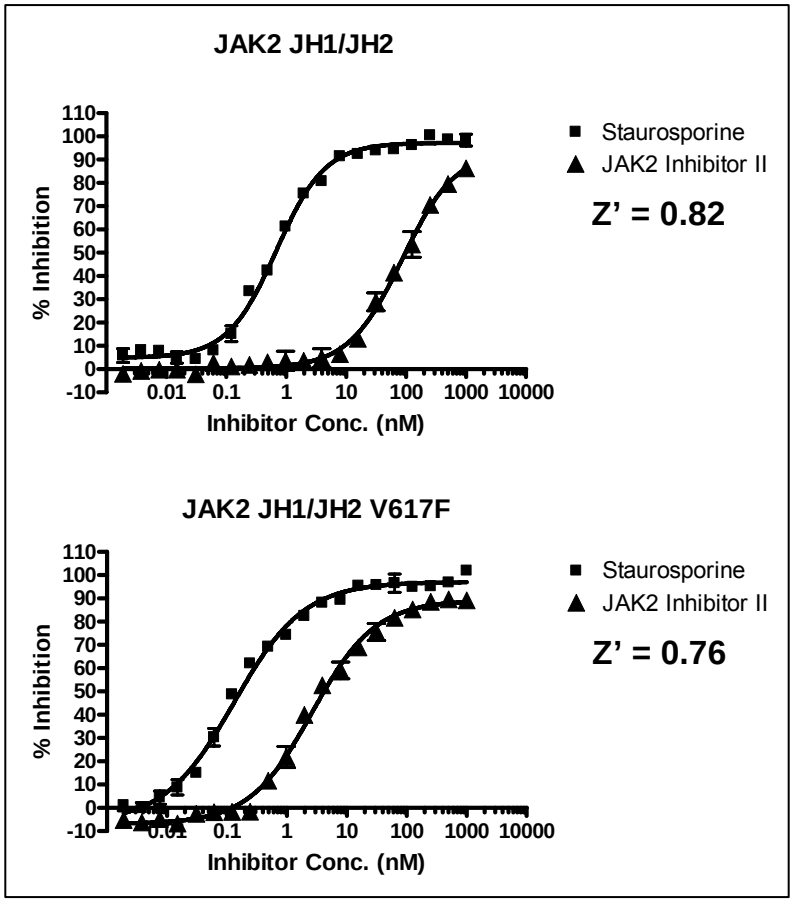


Figure 3: Adapta® Assay. Adapta® assay performed with the Tecan Safire²™.