Adapta® Assay Setup Guide on the BioTek Instruments Synergy™ 2 Multi-Mode Microplate Reader

NOTE: The BioTek Instruments Synergy™ 2 Multi-Mode 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 and provide representative data. 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 BioTek instruments or Gen5 software, please contact BioTek Instruments at 1-888-451-5171.

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).

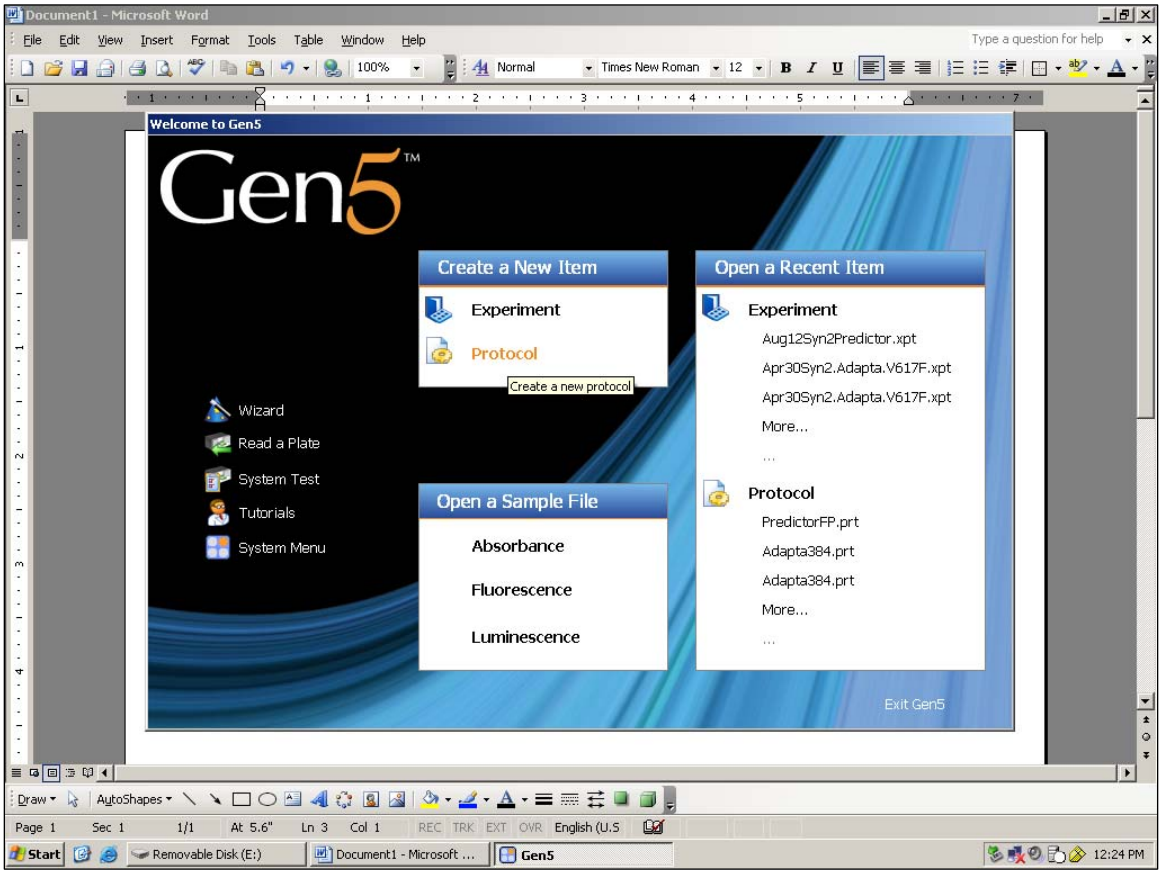
A. Recommended Optics

BioTek Instruments part number	wavelength (nm)	diameter (mm)
Excitation (7082230)	340/30	18
Emission 1 (7082265)	620/10	18
Emission 2 (7082266)	665/8	18
Dichroic Mirror (7138400)	400	

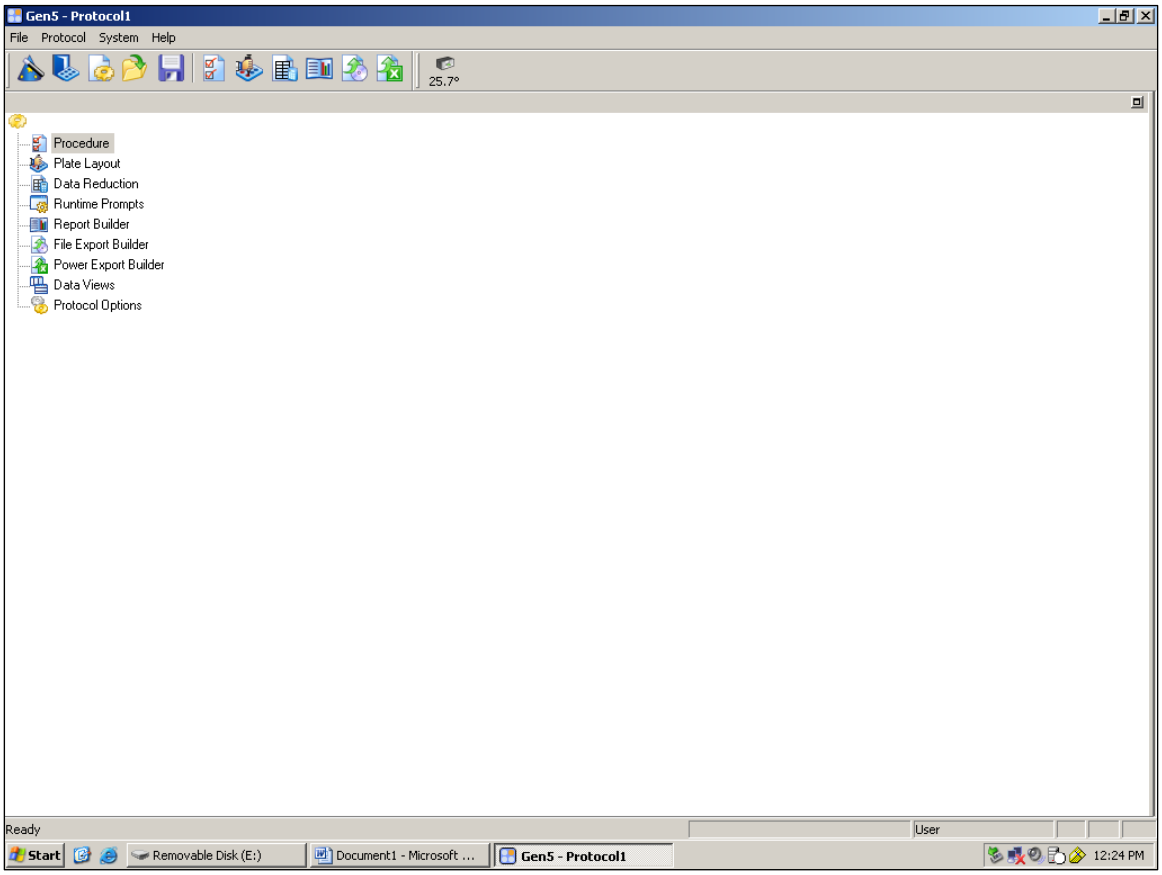
B. Instrument Setup

1. Make certain plate reader is turned on, and open up BioTek Gen5 software on computer.

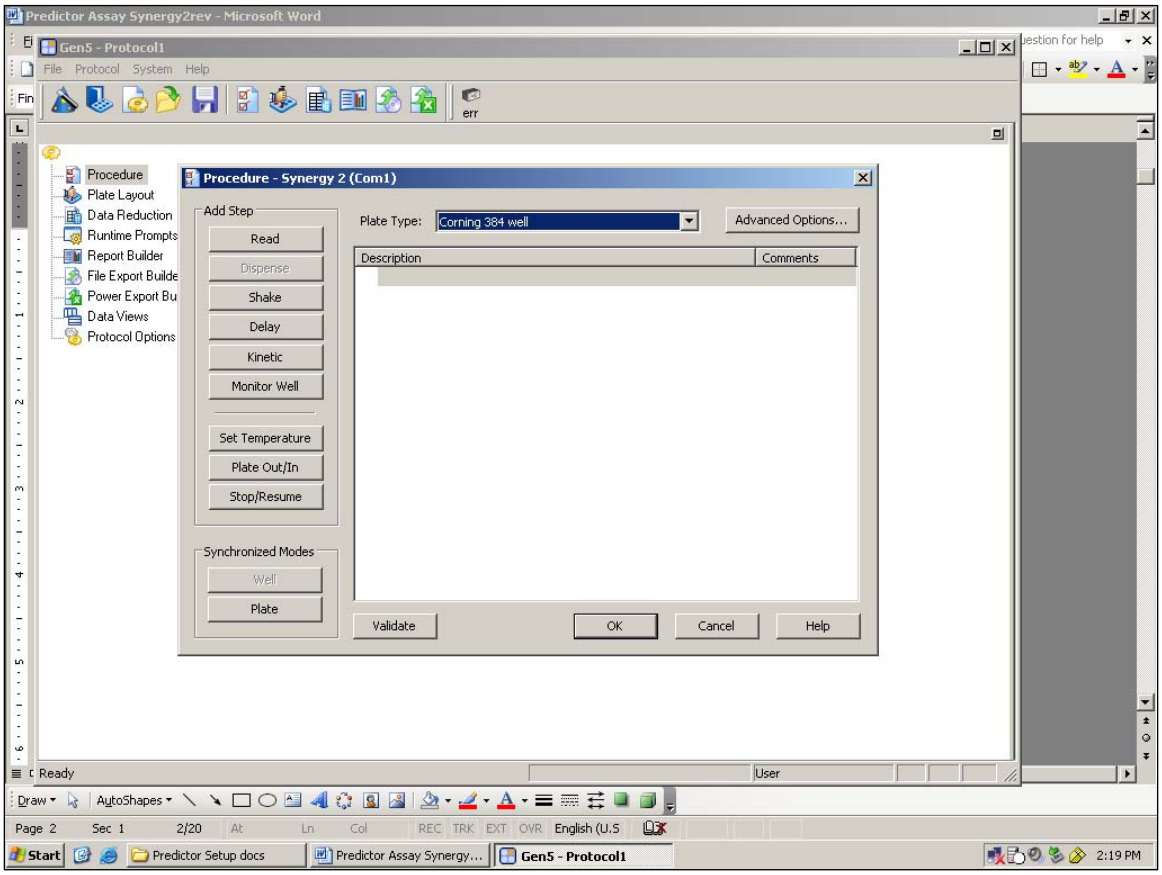
2. When Gen5 software opens, if you do not have a pre-existing protocol for Adapta®, select "Protocol" from the “Create a New Item” menu.



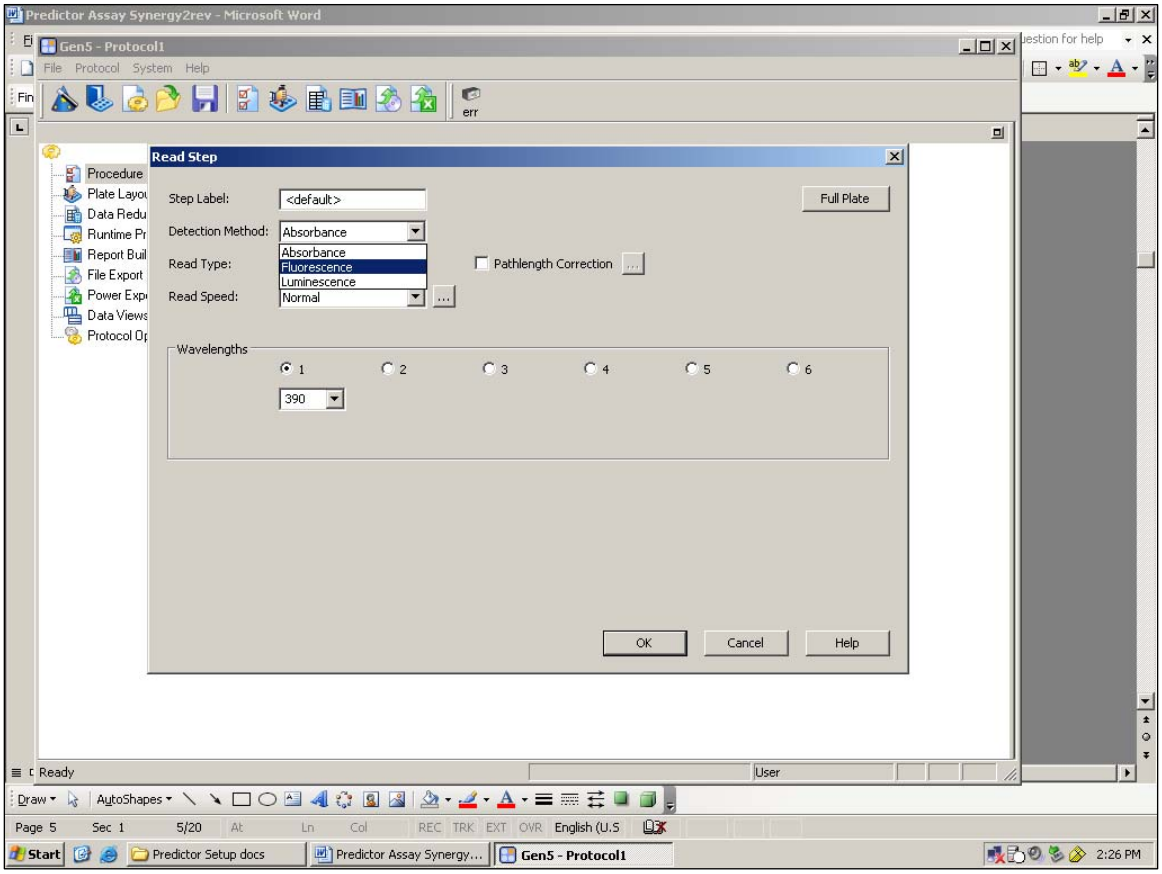
3. At this point, a new screen will open (below). Click on “Procedure” from the menu on the left side of the screen.



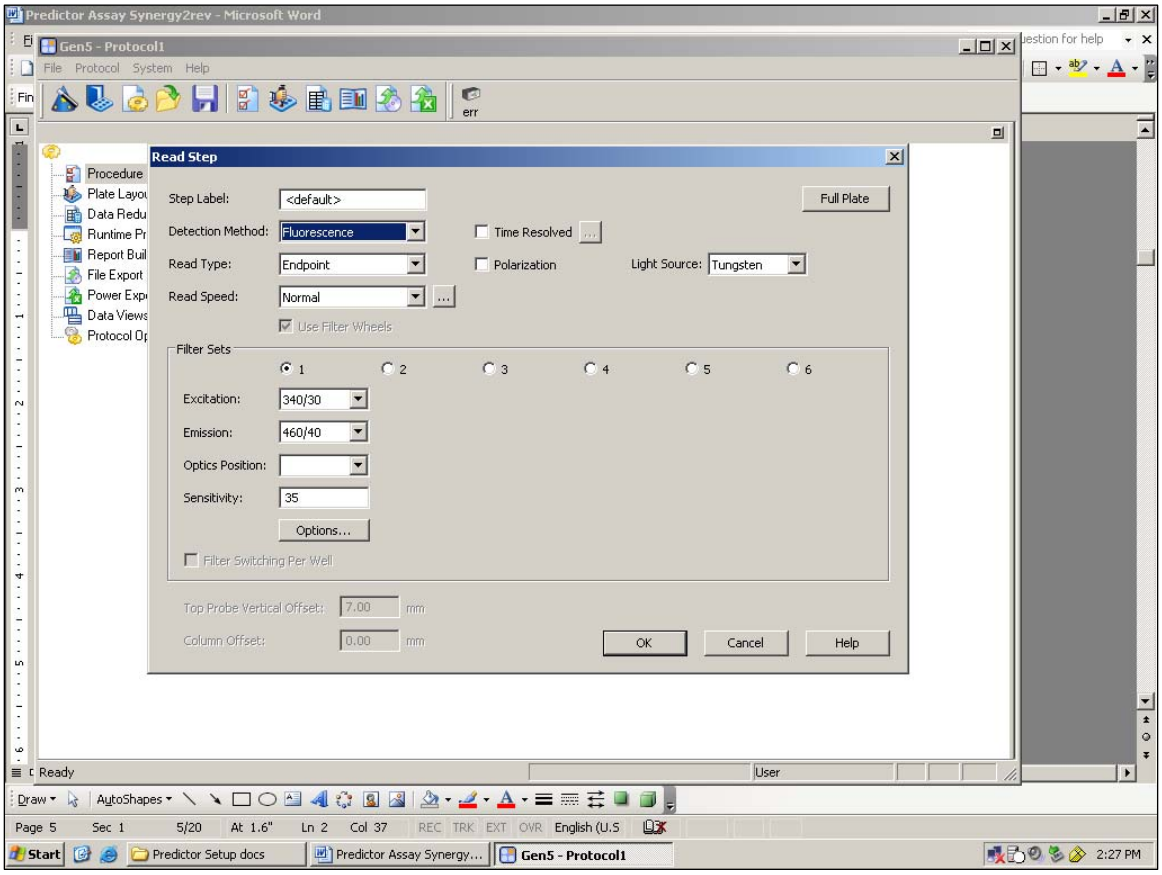
4. A new Procedure window will pop up. Select plate type from the drop-down menu. Next, click “Read” at the left side of the Procedure window.



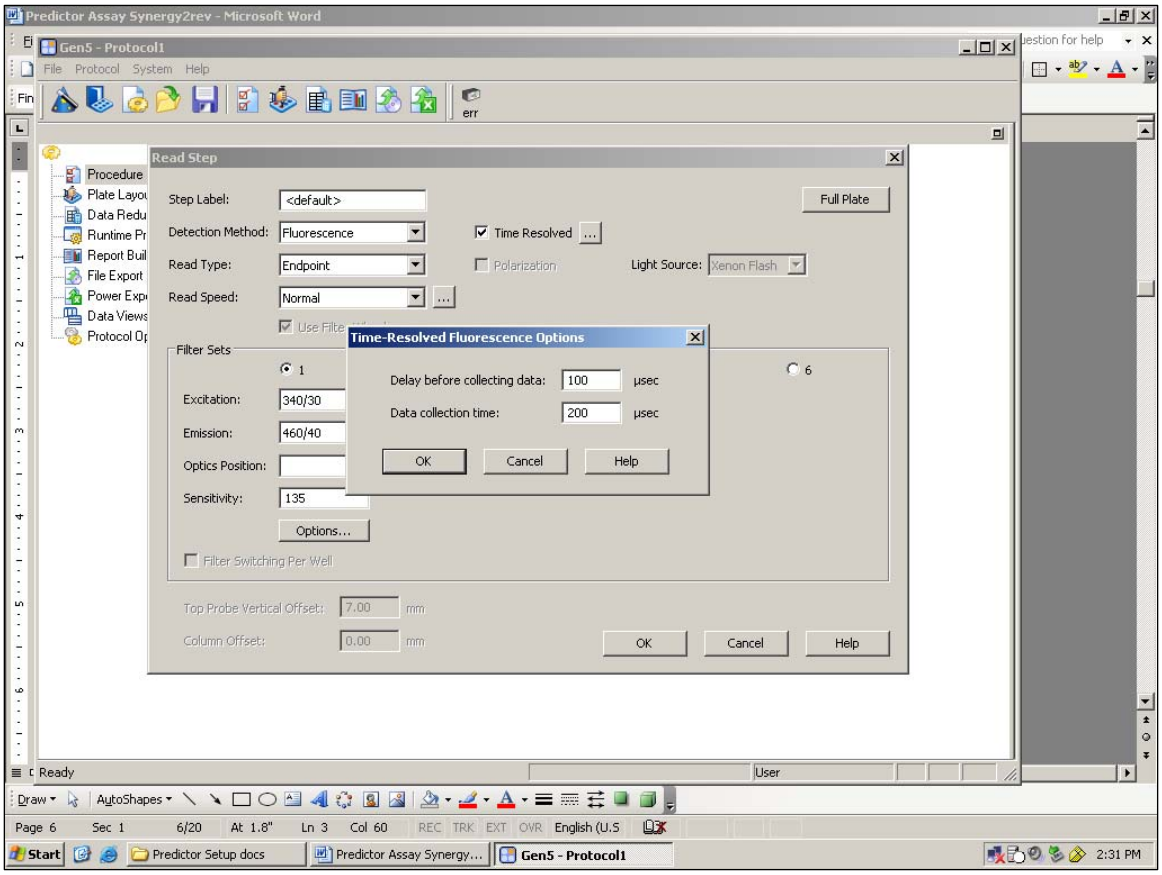
5. A “Read Step” window will open automatically. From the drop-down “Detection Method” window, select Fluorescence.



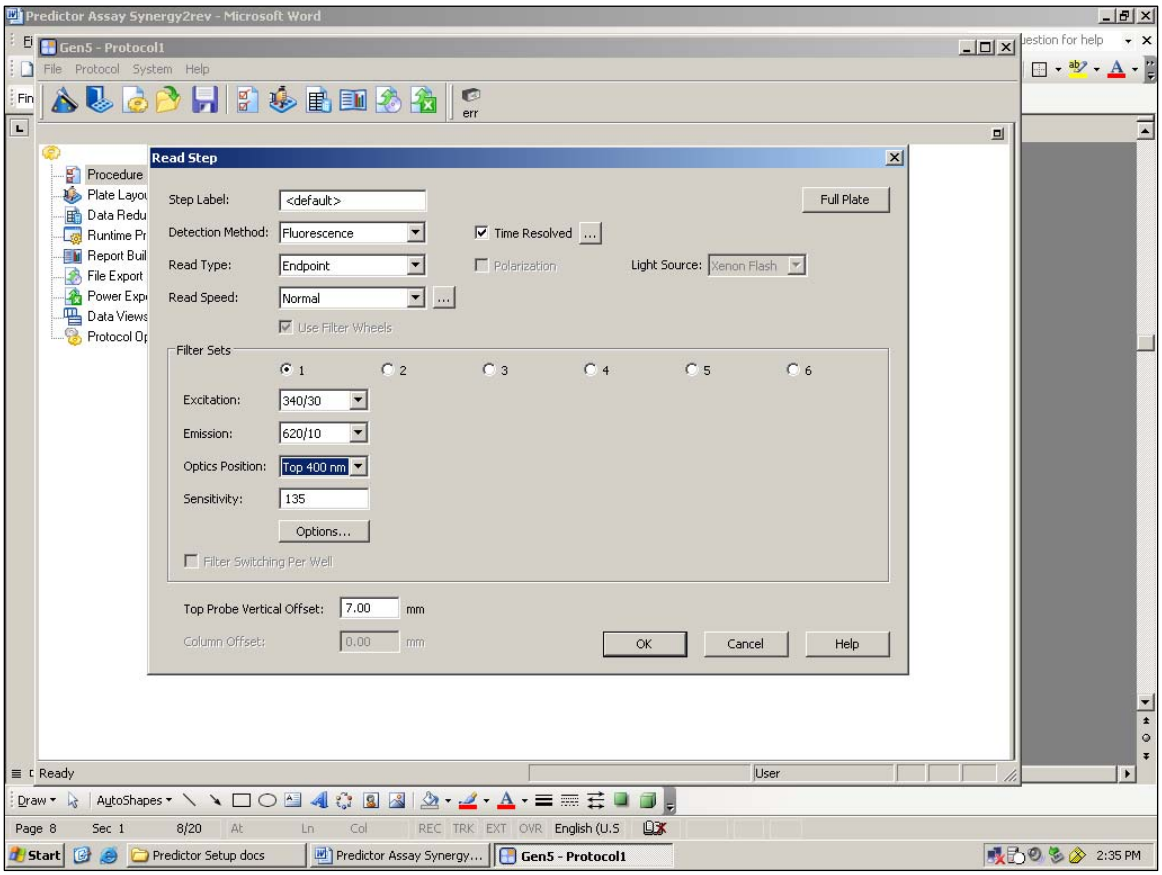
6. Once fluorescence is selected, the window will change and open a variety of other parameters. First, check the "Time-Resolved" box and then click on the icon with 3 dots to the right or the words "Time Resolved".



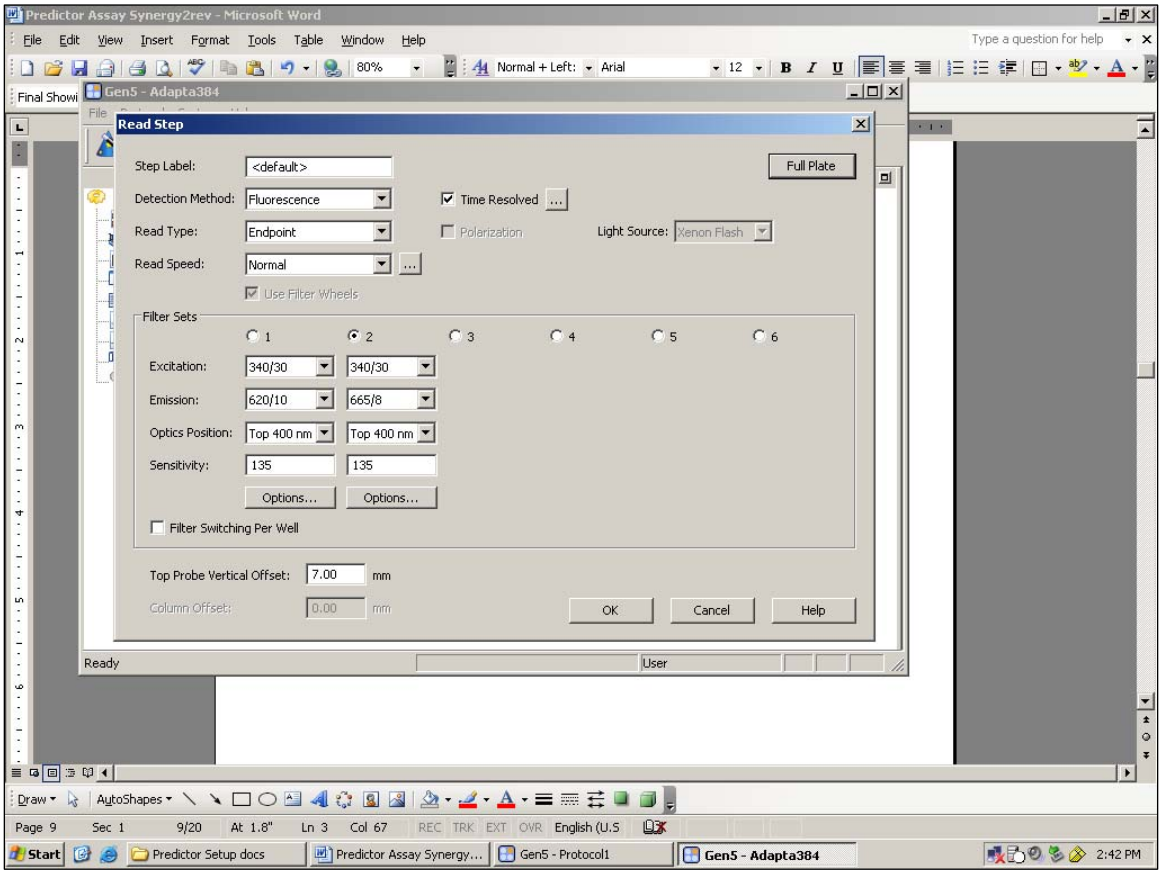
7. A new window will appear, allowing you to set the delay and integration times ("Delay before collecting data" and "Data collection time", respectively. Set at 100 and 200 µs. When finished, click on "OK".



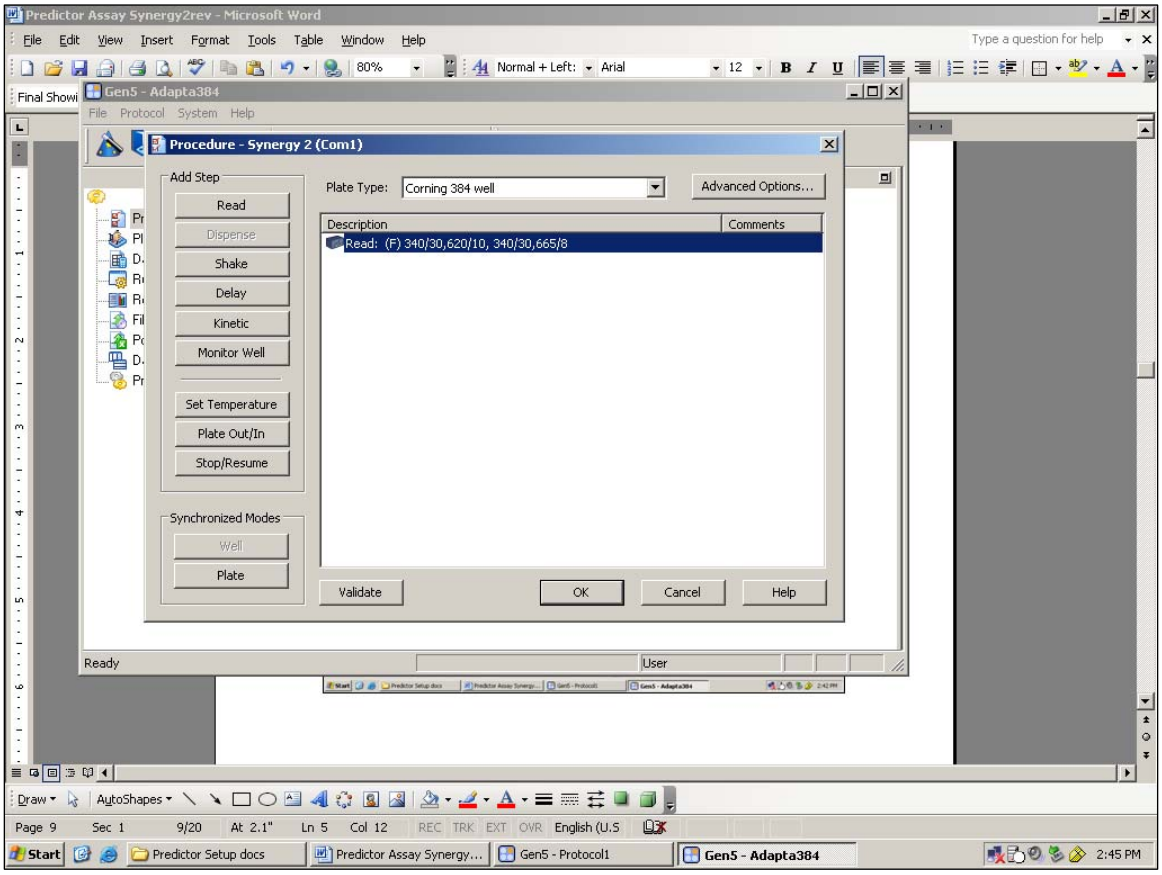
8. You will now return to the Read Step window. Select the appropriate filter sets and dichroic mirror ("Optics Position") from the drop-down menus. Sensitivity will default to a predetermined value, which can be manually re-set if needed, or calibrated automatically if desired by slicking the "Options" tab below Sensitivity).



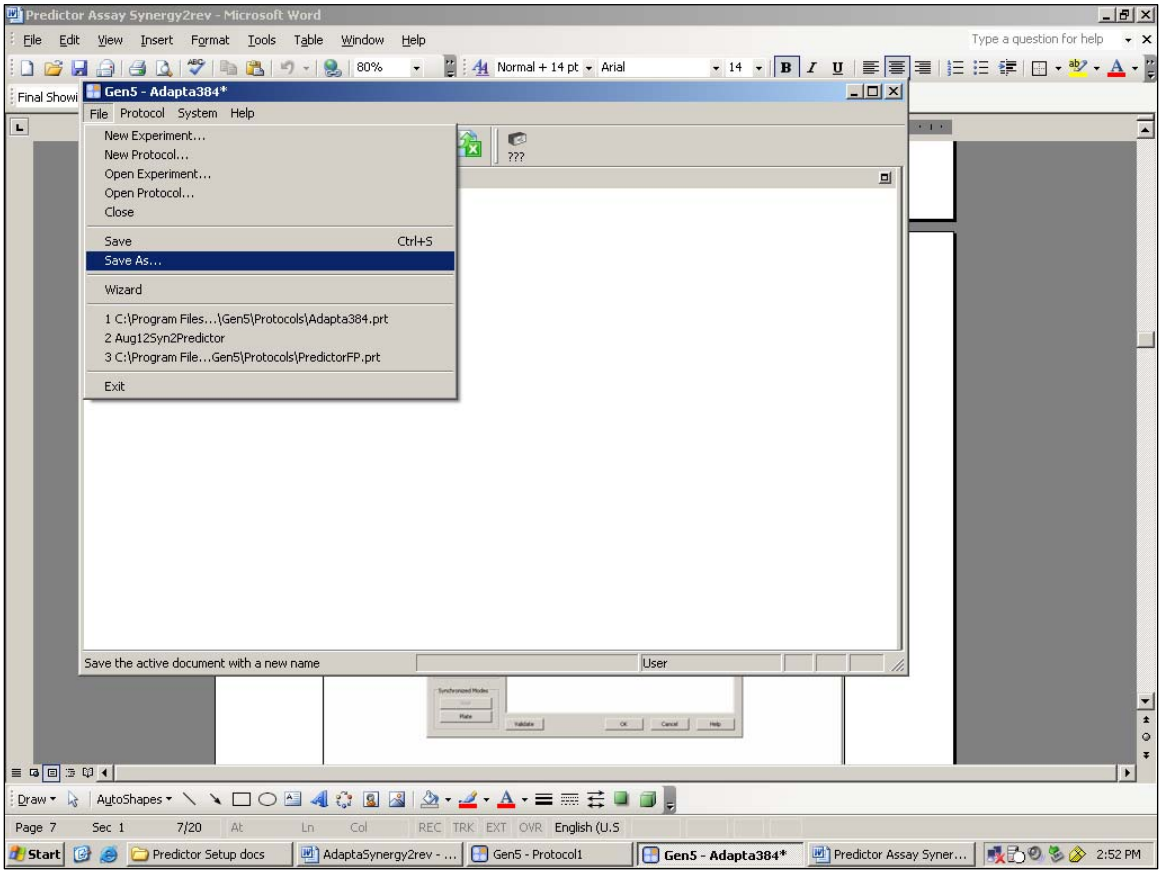
9. Click on the button #2 below Filter Sets and add parameters for your second fluorescent measurement (Adapta® is a ratiometric assay) and enter your parameters as below. If you wish to only read part of the plate, click on the "Full Plate" tab in the upper right to change the wells to be read now. When finished, click "OK".



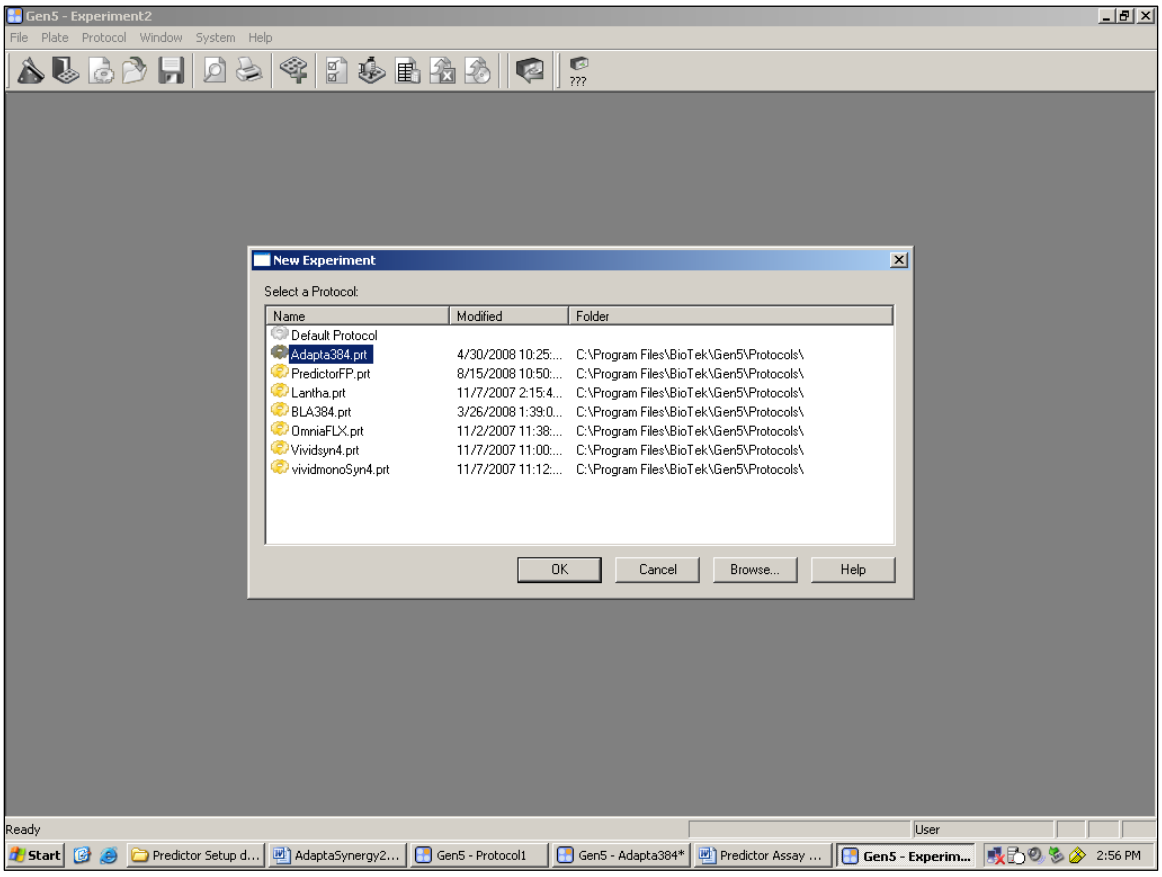
10. You will close the prior window and return to the Procedure window. Select "Validate" on the bottom of the screen. The software will verify that the instrument has the correct filters and that the assay parameters selected are valid, and a prompt will appear indicating the sequence is valid. Select "OK" on the popup window, and then select "OK" at the bottom of the Procedure window.



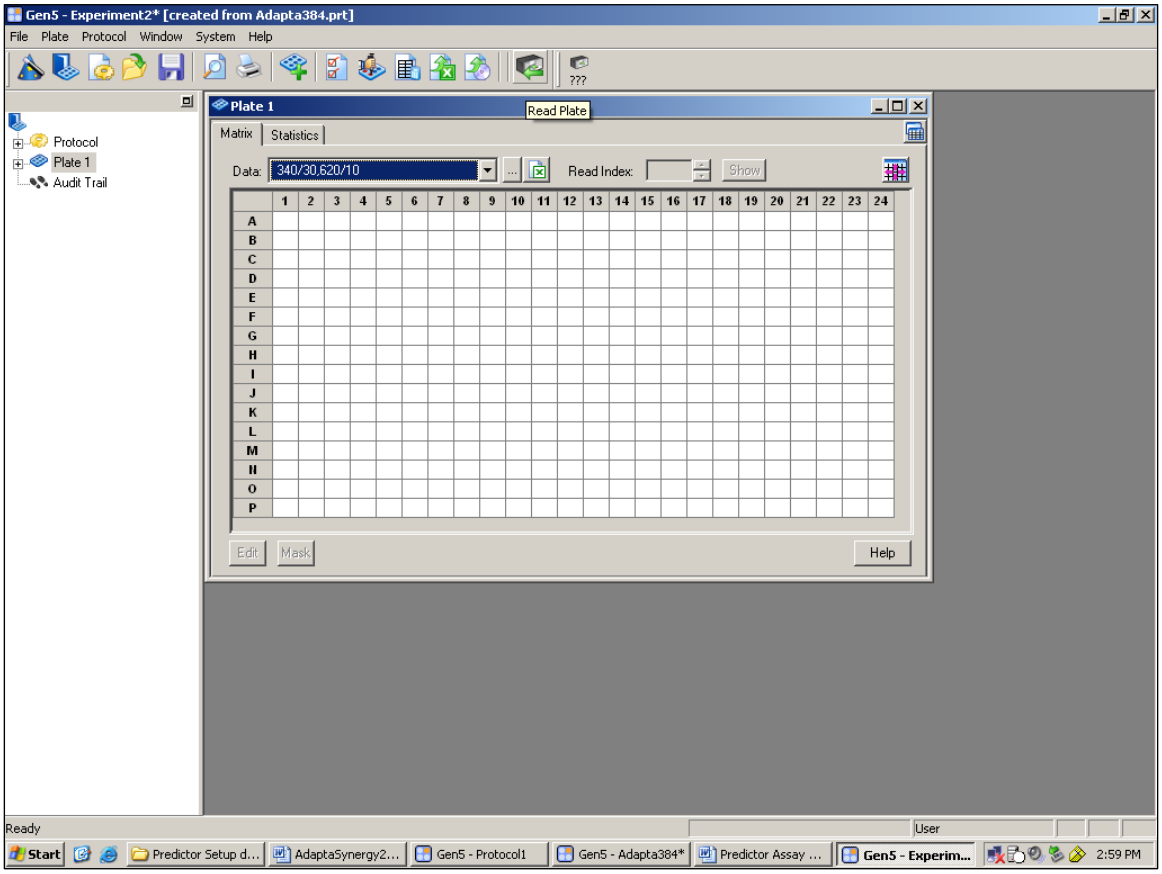
11. If you have not already, at this time in the Gen5 menu at the top, go to “File” and then “Save As”. Save your new protocol.



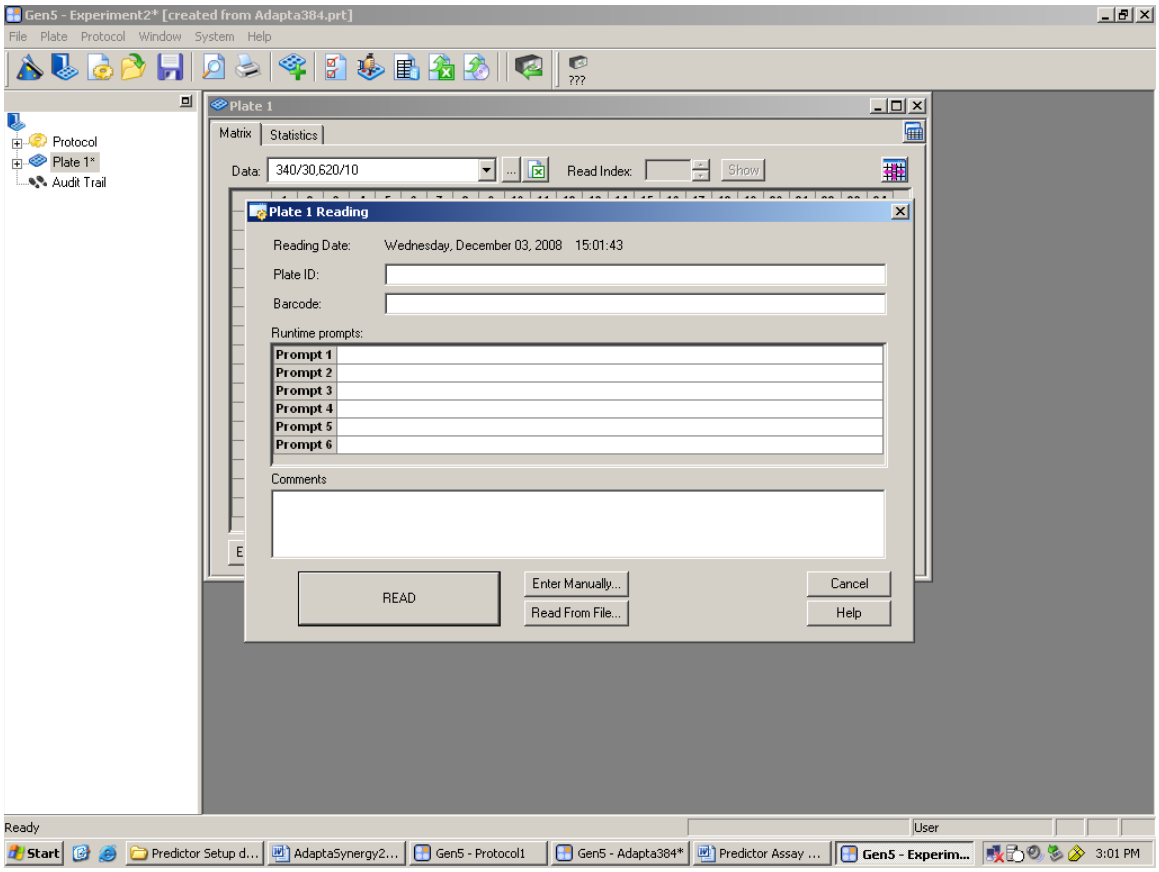
12. Again under “File”, select “New Experiment”. Select your protocol from the list, and select “OK”.



13. A new Read Step window will appear. Select the “Read Plate” icon at the top of the window.

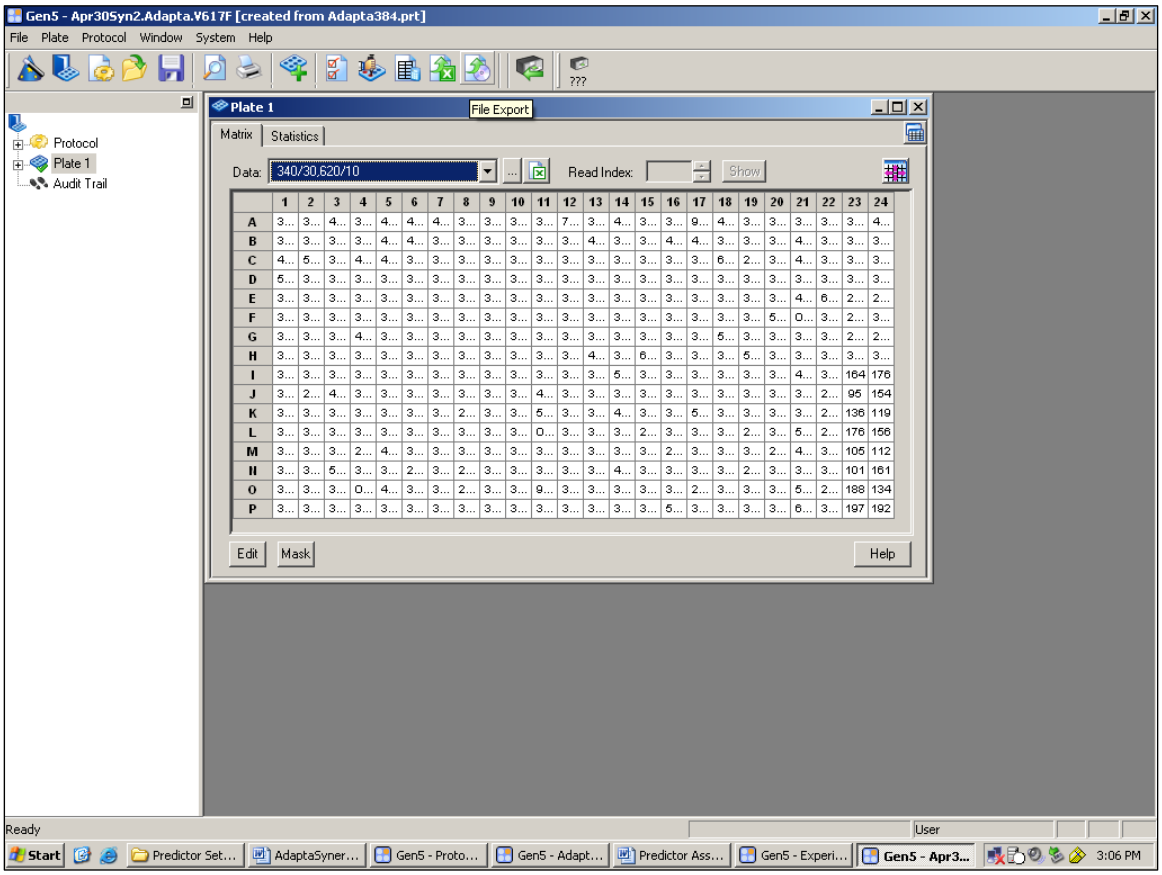


14. A second window will appear. Select "Read" again. A final prompt will appear instructing you to place your plate on the carrier and select OK when ready. Selecting OK will load the plate and begin the assay readout.



15. A final prompt will appear. Press “OK” to initiate reading.

16. Synergy will commence reading of plate. As the instrument reads, data will begin to populate the cells as shown below. Data can be exported when finished by selecting the “Export” button or by preparing formal export settings in the export wizard.



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.

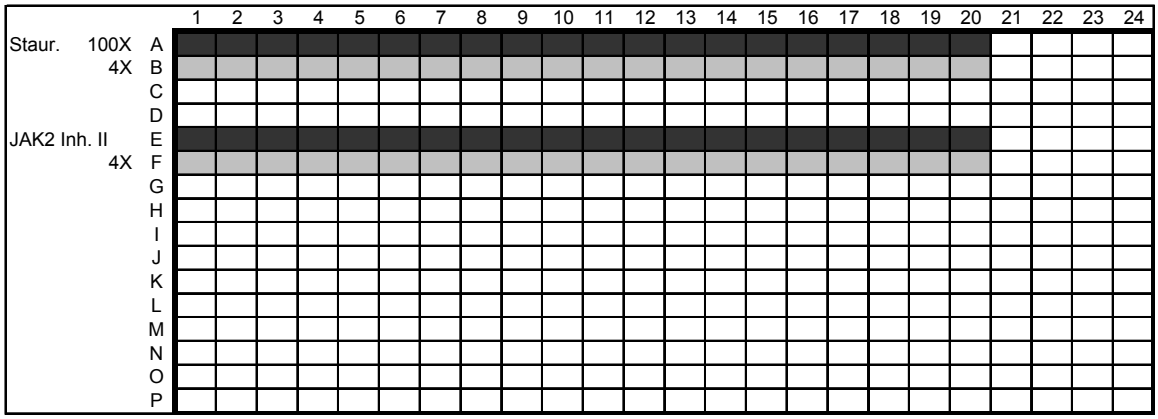


Figure 1: Schematic of initial compound dilution. Staurosporine and JAK2 Inhibitor II were titrated from a 100 µM starting concentration in the initial dilution series by preparing a 1:1 dilution curve in DMSO. A secondary dilution to 4X was then prepared in the rows below the initial dilution curve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

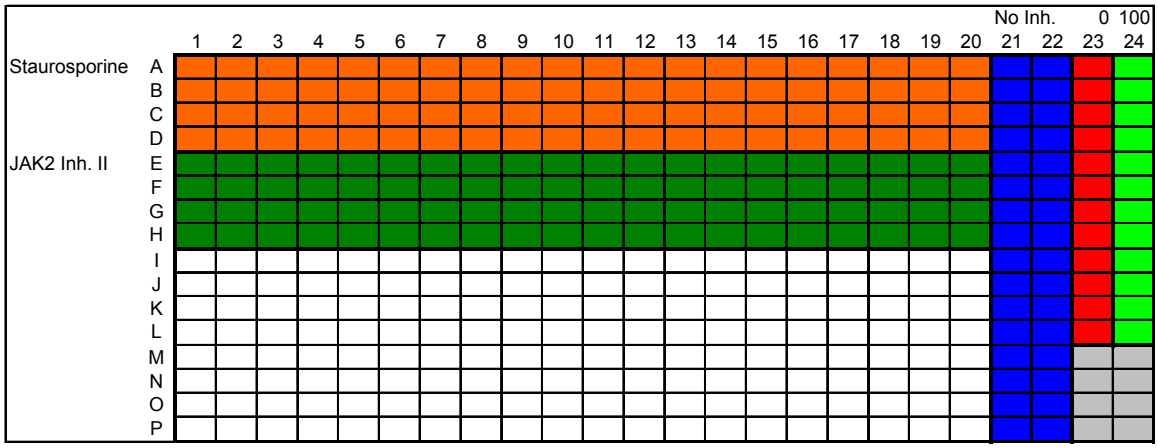


Figure 2: Assay Plate Schematic. Compound titrations shown in Columns 1-20, Columns 21 and 22 prepared without any inhibitor as kinase activity controls, Column 23 prepared with no kinase (0% phosphorylation) and Column 24 prepared using 20% ADP as a positive control (100% phosphorylation). Note 8 wells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

D. Results:

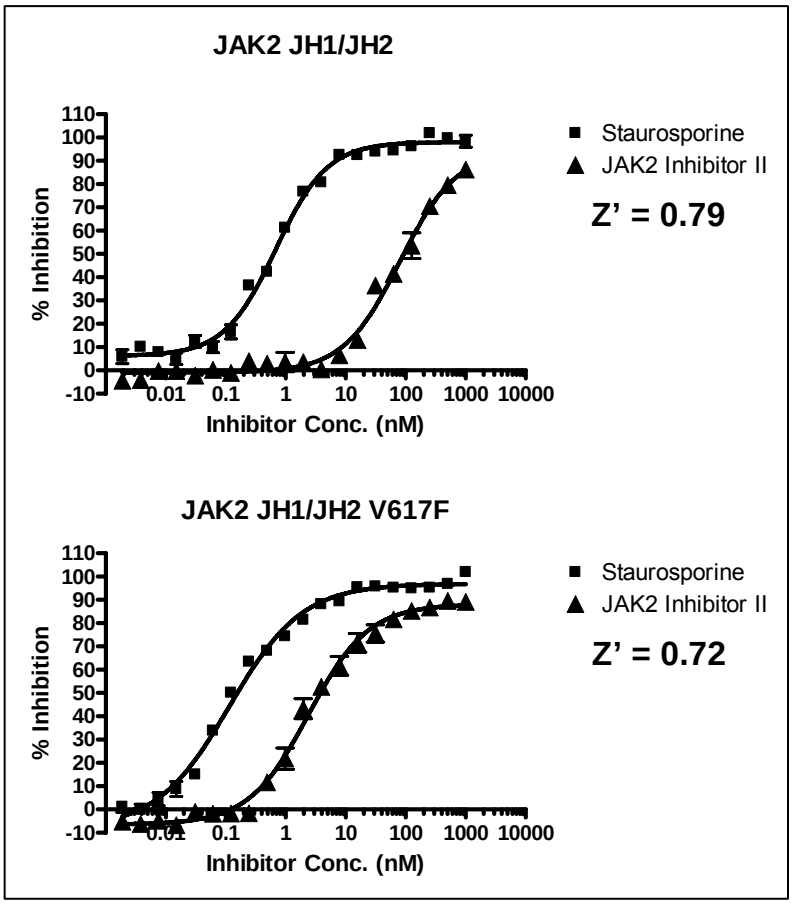


Figure 3: Adapta® Assay. Adapta® assay performed with the BioTek Instruments Synergy™ 2.