# Z'-LYTE® Assay Setup Guide on the BMG LABTECH PHERAstar/PHERAstar*Plus* Microplate Readers

NOTE: The BMG LABTECH PHERAstar/PHERAstar*Plus* Microplate Readers were tested for compatibility with Invitrogen's Z'-LYTE® Assay using the Z'-LYTE® Tyr6 kit (PV4122) 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 BMG LABTECH instruments or software, please contact BMG LABTECH at 1-877-264-5227 or www.bmglabtech.com.

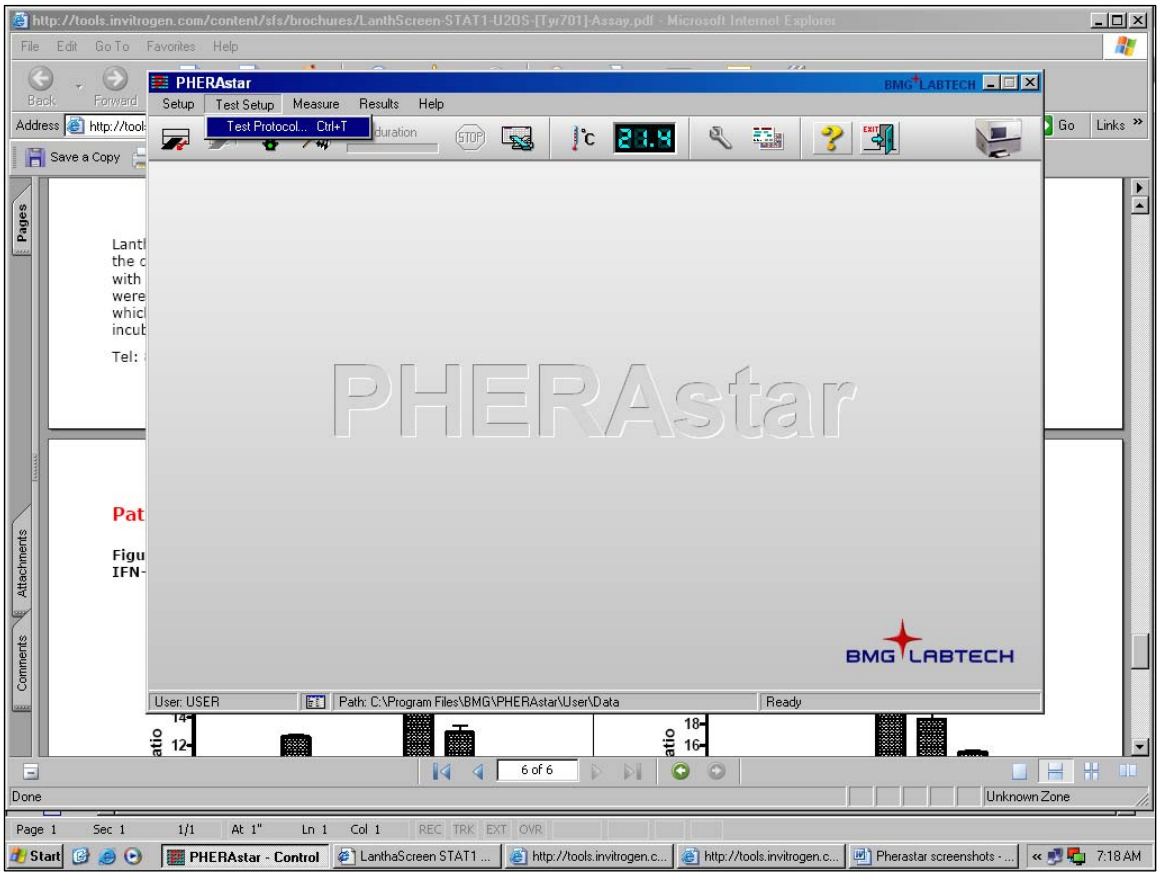
## A. Recommended Optics

| | wavelength (nm) | BMG LABTECH Optic Module |
|---|---|---|
| Excitation | 400 (or similar) | *contact BMG LABTECH |
| Emission 1 | 440 (or similar) | *contact BMG LABTECH |
| Emission 2 | 520 (or similar) | *contact BMG LABTECH |
| Dichroic Mirror | | *contact BMG LABTECH |

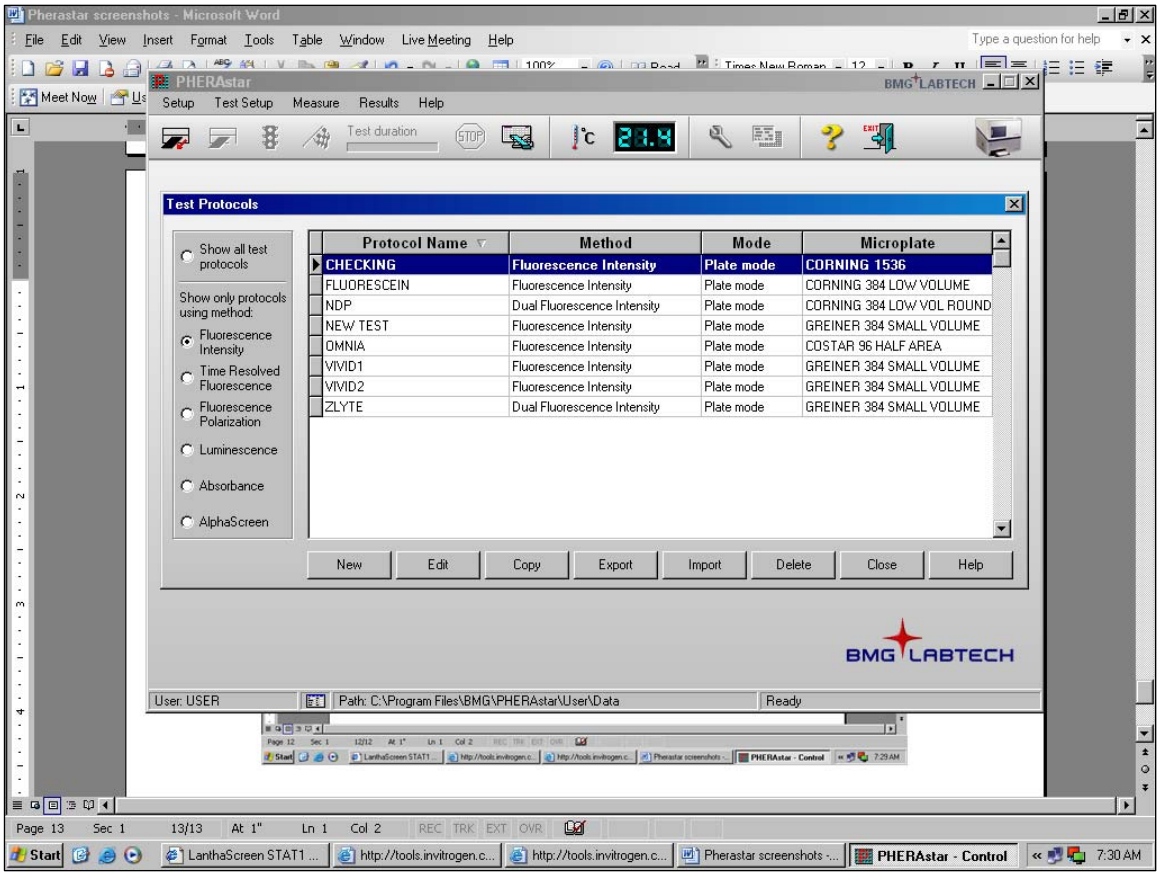
## B. Instrument Setup

1. Make certain plate reader is turned on, and open up PHERAstar Control software on computer. Insert plate into plate reader.

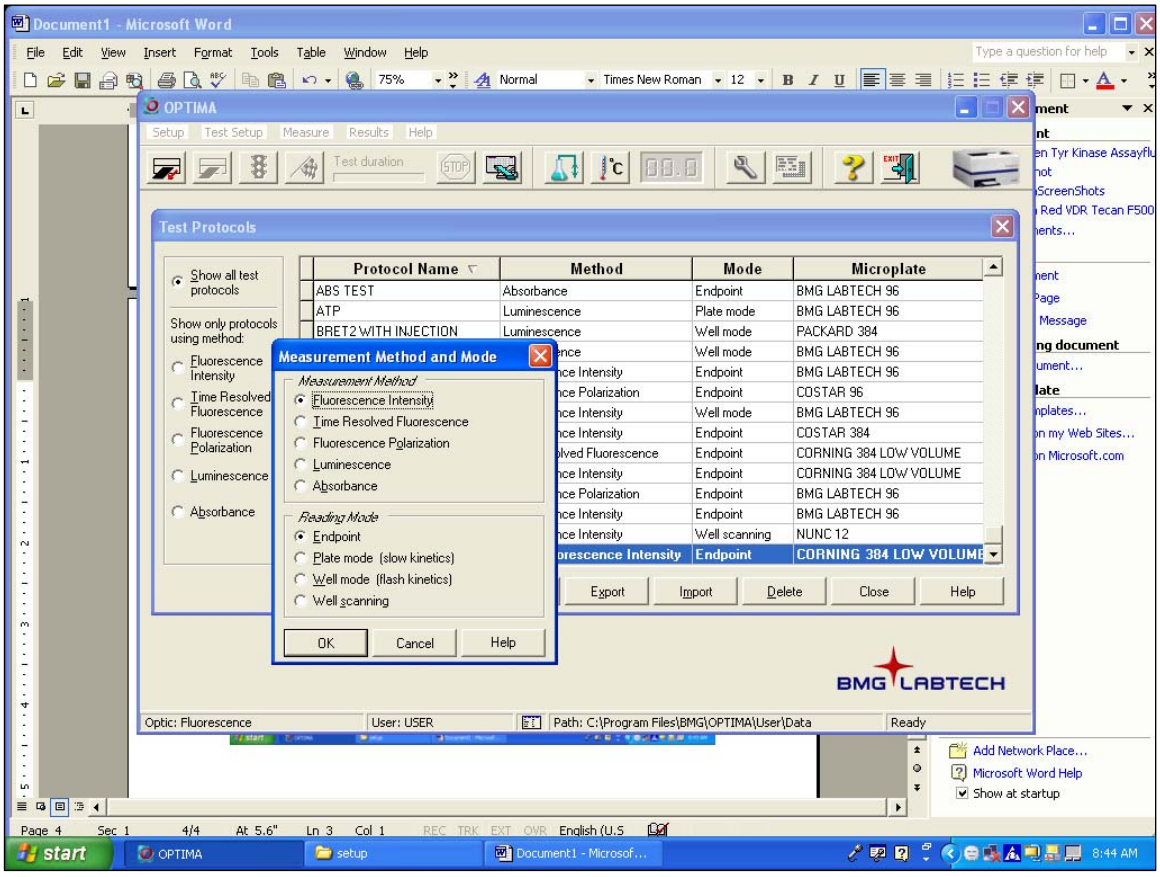
2. Setup protocol for Z'-LYTE®, select "Test Protocol" from the Test Setup menu bar at the top of the window.



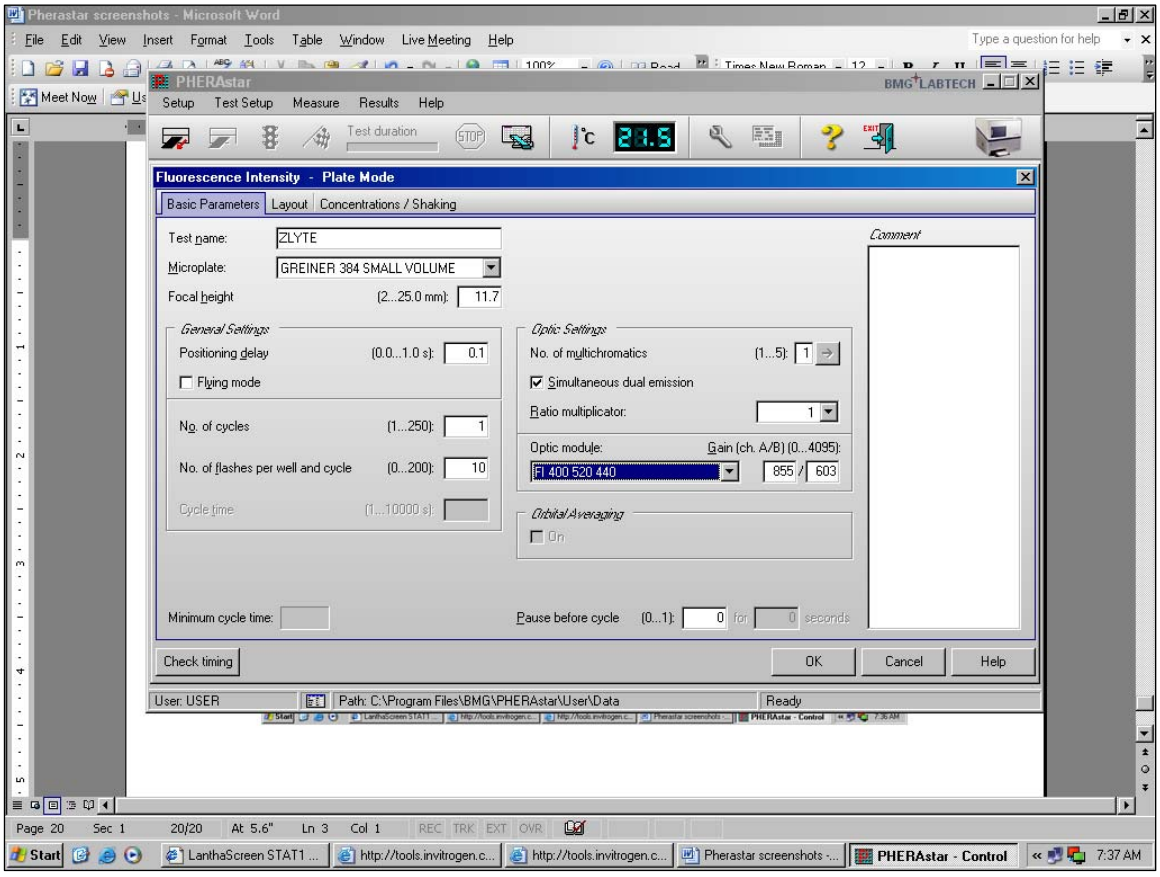
3. At this point, a new screen will open (below). Click on the "Show all test protocols" or "Fluorescence Intensity" button on the left side of the screen, then select "New" from the tabs at the bottom.



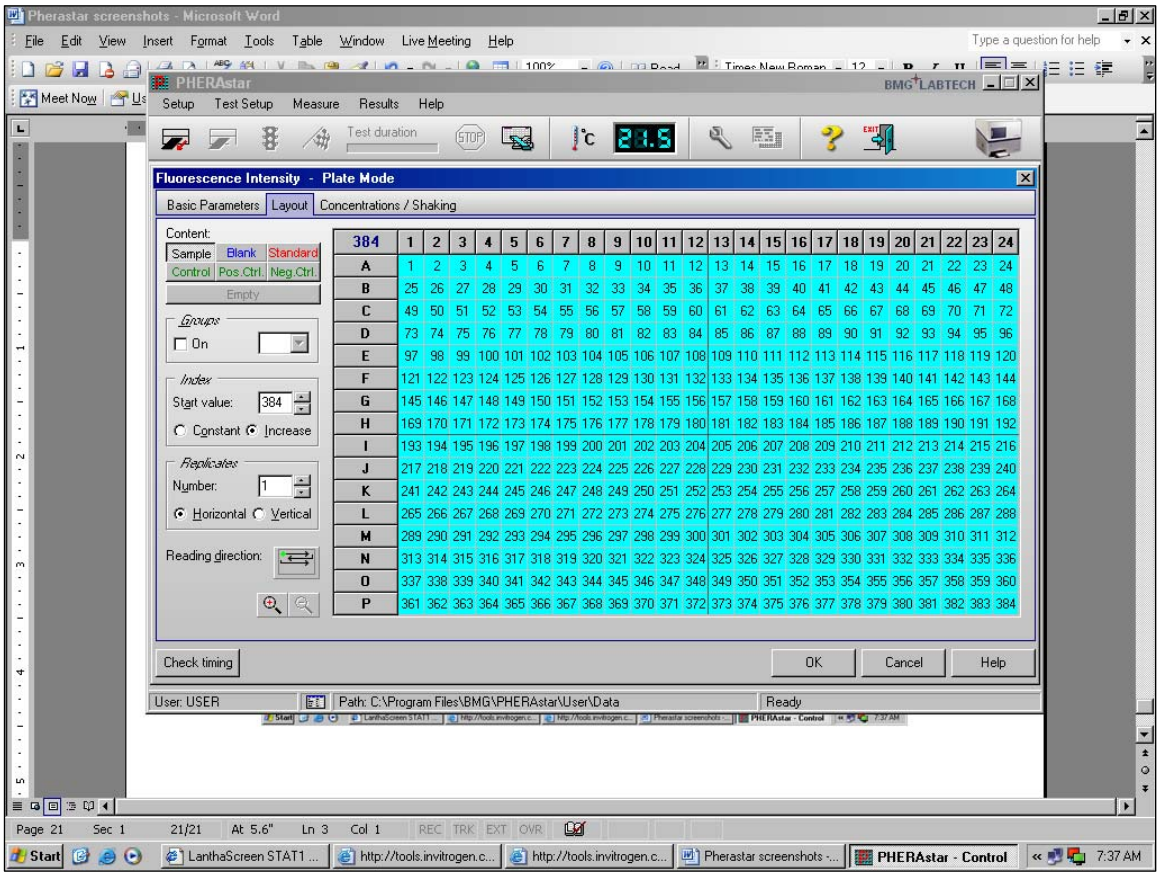
4. A new window will pop up. Select "Fluorescence Intensity" and "Endpoint" and then select "OK".



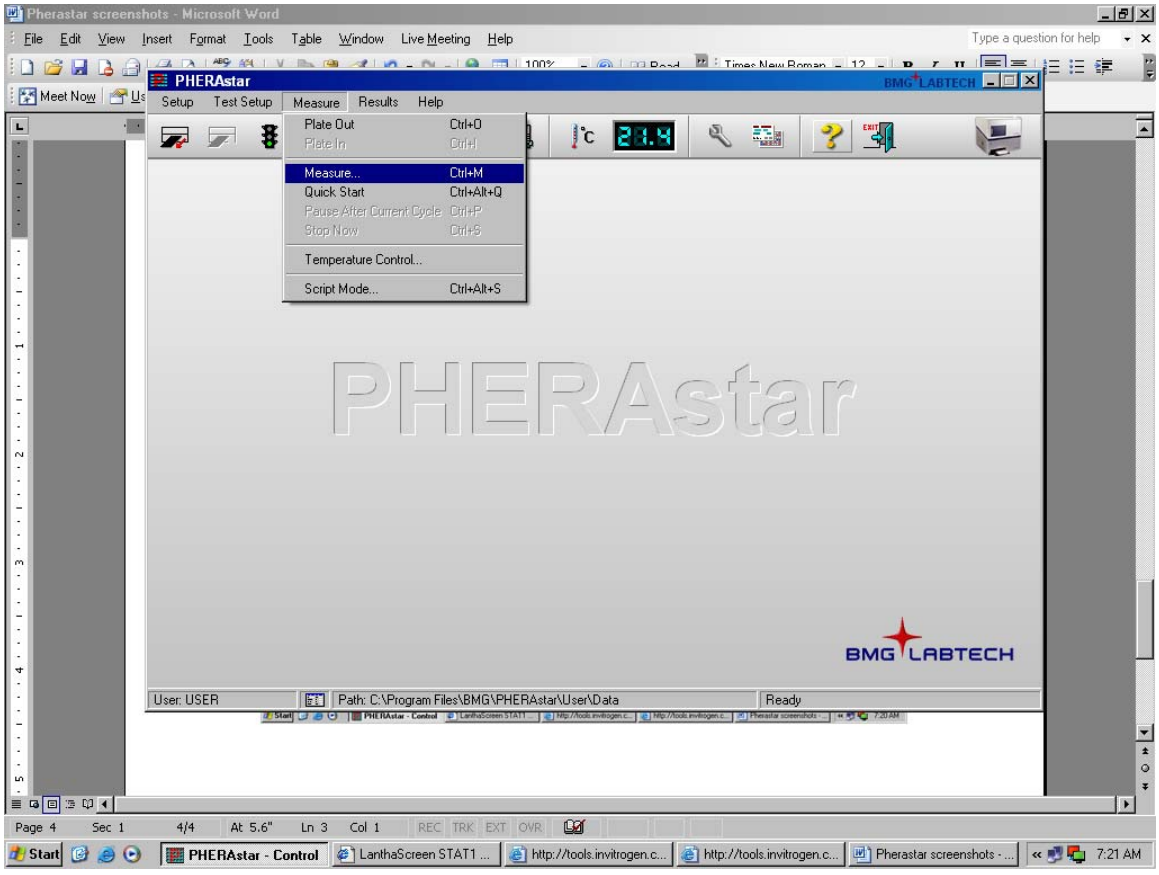
5. A new Protocol window will open automatically. Enter a test name, select plate type, and check the "Simultaneous Dual Emission" box. From the drop-down menu, select your optic module. When finished, select the "Layout" tab at the top of the Protocol window.



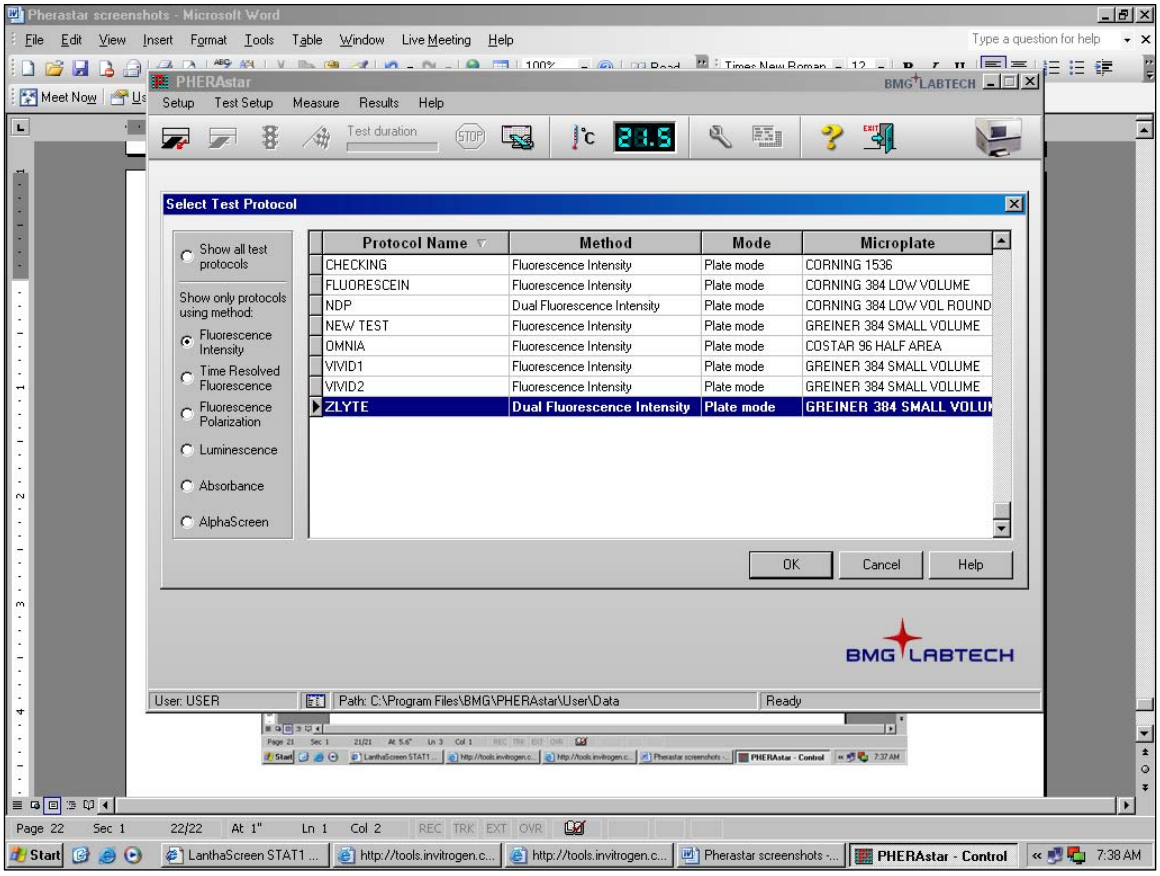
6. Select the wells you wish to read. Note in this step you can select to designate blanks, positive controls, etc. but for this case we marked all wells "Sample" and calculations were performed manually. When finished, select OK.



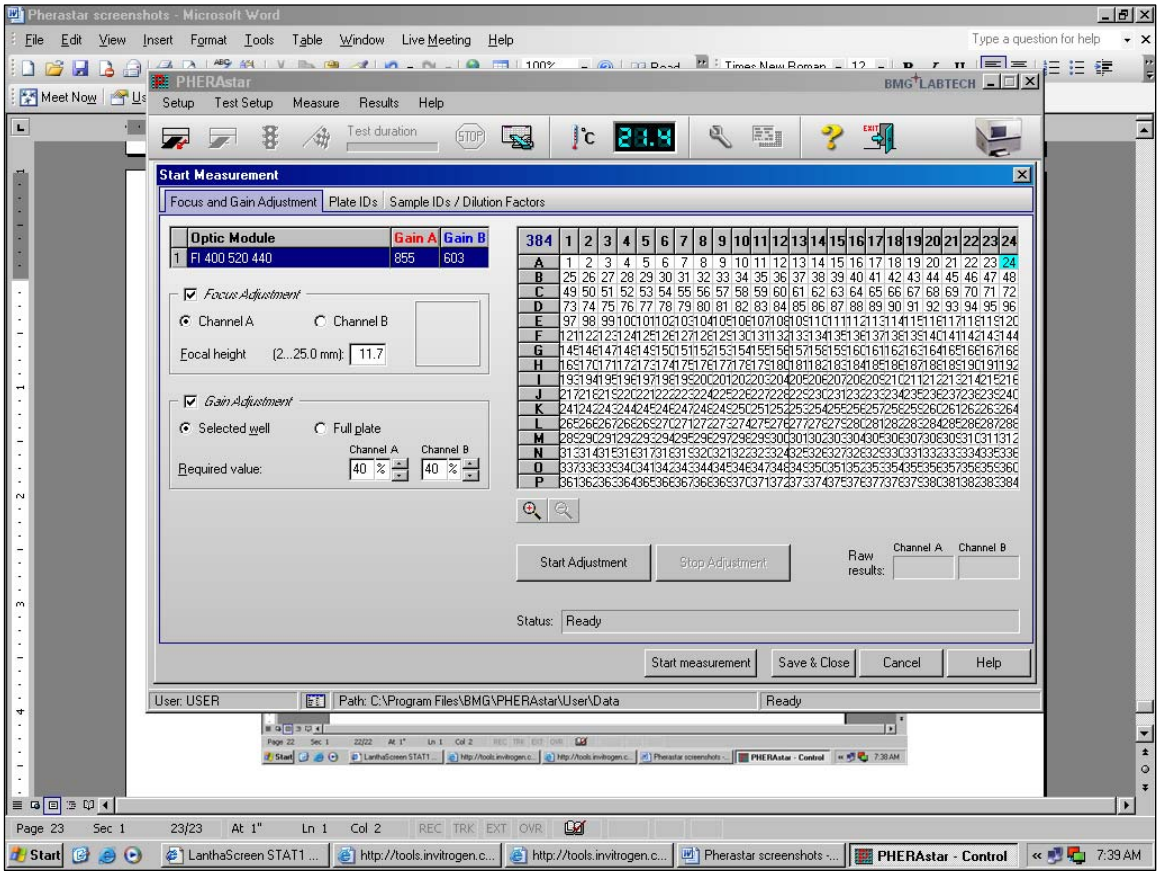
7. You will return to the initial settings window. From the drop-down menus at the top, select "Measure" and "Measure" again.



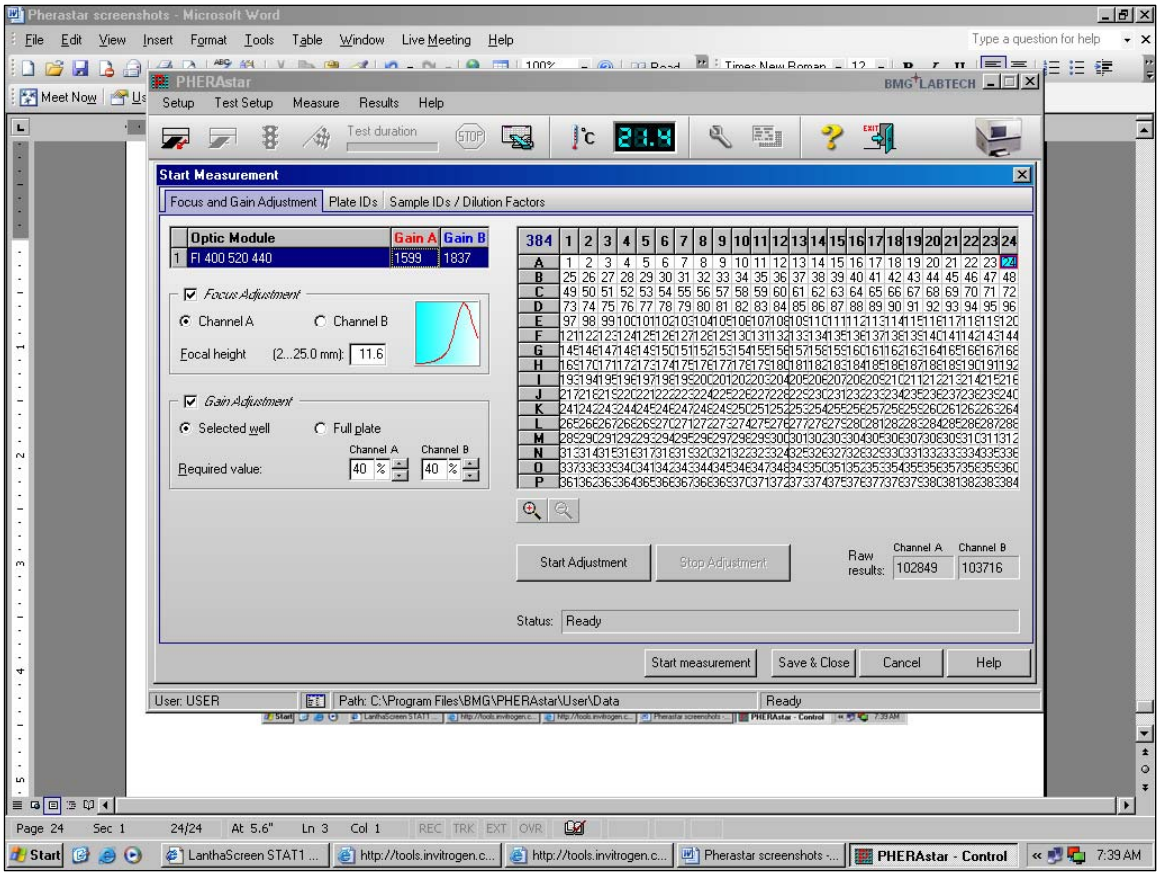
8. A new window will appear allowing you to select which of your test protocols you wish to run. Select the protocol you created for Z'-LYTE®, and then press OK.



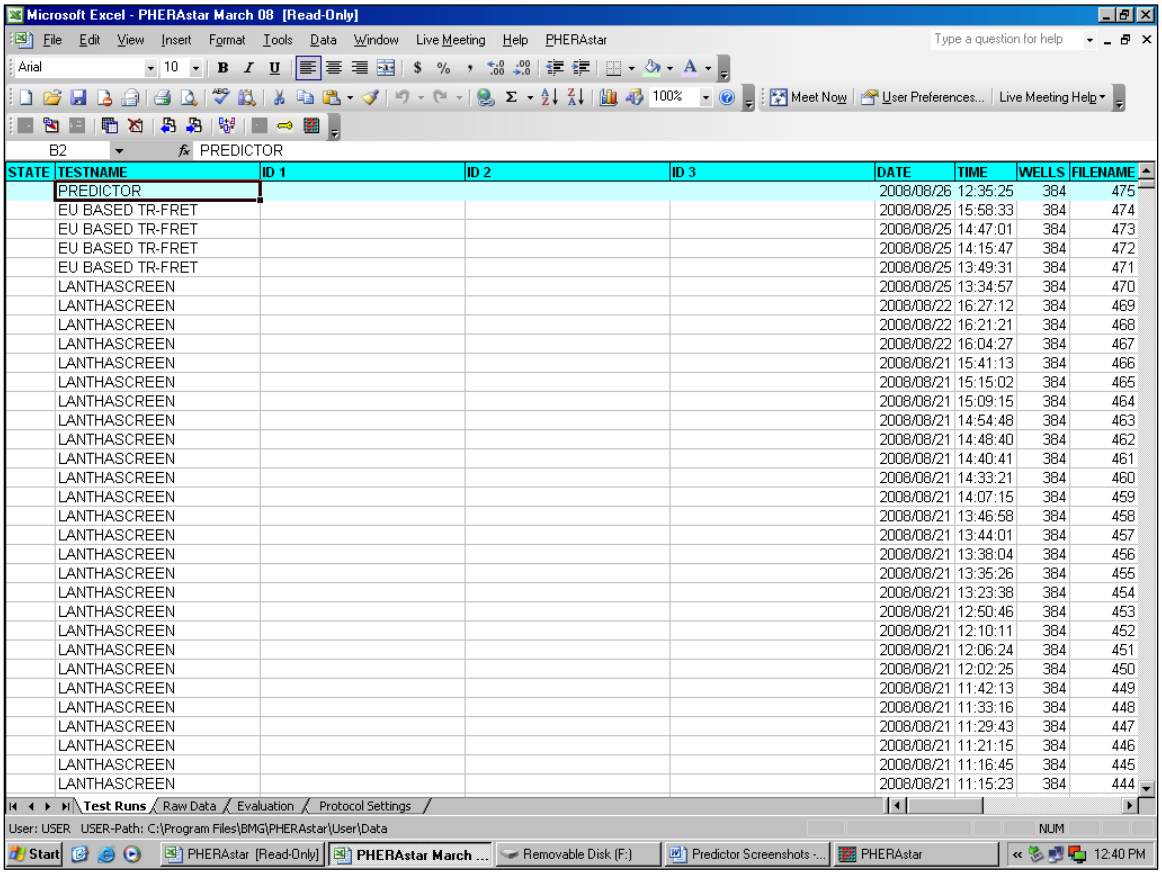
9. A new window will appear. Place your plate in the reader, and select a well to use for adjusting gain and focus by highlighting the well of your choice. The gain or sensitivity can be adjusted for each channel at this point, in this case a positive control (high FRET) well was used. When finished, click on the "Start Adjustment" tab.



10. In a moment, the instrument will have calculated it's optimal focal height and the gain adjustments necessary. Wehn finished, click on the "Start Measurement" tab to read.



11. When PHERAstar is done reading, you can collect your data by clicking "Results" on the toolbar at the top of the window. This will automatically redirect you to a Microsoft Excel file which collects run data. Select your run of interest from the list to open, and then select the "Raw Data" tab at the bottom to view data in a plate layout format.




| STATE | TESTNAME | ID 1 | ID 2 | ID 3 | DATE | TIME | WELLS | FILENAME |
|---|---|---|---|---|---|---|---|---|
| | PREDICTOR | | | | 2008/08/26 | 12:35:25 | 384 | 475 |
| | EU BASED TR-FRET | | | | 2008/08/25 | 15:58:33 | 384 | 474 |
| | EU BASED TR-FRET | | | | 2008/08/25 | 14:47:01 | 384 | 473 |
| | EU BASED TR-FRET | | | | 2008/08/25 | 14:15:47 | 384 | 472 |
| | EU BASED TR-FRET | | | | 2008/08/25 | 13:49:31 | 384 | 471 |
| | LANTHASCREEN | | | | 2008/08/25 | 13:34:57 | 384 | 470 |
| | LANTHASCREEN | | | | 2008/08/22 | 16:27:12 | 384 | 469 |
| | LANTHASCREEN | | | | 2008/08/22 | 16:21:21 | 384 | 468 |
| | LANTHASCREEN | | | | 2008/08/22 | 16:04:27 | 384 | 467 |
| | LANTHASCREEN | | | | 2008/08/21 | 15:41:13 | 384 | 466 |
| | LANTHASCREEN | | | | 2008/08/21 | 15:15:02 | 384 | 465 |
| | LANTHASCREEN | | | | 2008/08/21 | 15:09:15 | 384 | 464 |
| | LANTHASCREEN | | | | 2008/08/21 | 14:54:48 | 384 | 463 |
| | LANTHASCREEN | | | | 2008/08/21 | 14:48:40 | 384 | 462 |
| | LANTHASCREEN | | | | 2008/08/21 | 14:40:41 | 384 | 461 |
| | LANTHASCREEN | | | | 2008/08/21 | 14:33:21 | 384 | 460 |
| | LANTHASCREEN | | | | 2008/08/21 | 14:07:15 | 384 | 459 |
| | LANTHASCREEN | | | | 2008/08/21 | 13:46:58 | 384 | 458 |
| | LANTHASCREEN | | | | 2008/08/21 | 13:44:01 | 384 | 457 |
| | LANTHASCREEN | | | | 2008/08/21 | 13:38:04 | 384 | 456 |
| | LANTHASCREEN | | | | 2008/08/21 | 13:35:26 | 384 | 455 |
| | LANTHASCREEN | | | | 2008/08/21 | 13:23:38 | 384 | 454 |
| | LANTHASCREEN | | | | 2008/08/21 | 12:50:46 | 384 | 453 |
| | LANTHASCREEN | | | | 2008/08/21 | 12:10:11 | 384 | 452 |
| | LANTHASCREEN | | | | 2008/08/21 | 12:06:24 | 384 | 451 |
| | LANTHASCREEN | | | | 2008/08/21 | 12:02:25 | 384 | 450 |
| | LANTHASCREEN | | | | 2008/08/21 | 11:42:13 | 384 | 449 |
| | LANTHASCREEN | | | | 2008/08/21 | 11:33:16 | 384 | 448 |
| | LANTHASCREEN | | | | 2008/08/21 | 11:29:43 | 384 | 447 |
| | LANTHASCREEN | | | | 2008/08/21 | 11:21:15 | 384 | 446 |
| | LANTHASCREEN | | | | 2008/08/21 | 11:16:45 | 384 | 445 |
| | LANTHASCREEN | | | | 2008/08/21 | 11:15:23 | 384 | 444 |

## C. Z'-LYTE® Kinase Assay using JAK2 JH1/JH2 and JAK2 JH1/JH2 V617F

NOTE: The following is a sample assay performed for demonstration purposes. The section below describes how the data was obtained, and is not intended for use as an assay protocol. We recommend all first-time users follow the appropriate protocols and/or validation packets provided with their specific assay kits, and include all proper controls. The instrument settings above would be sufficient for any Z'-LYTE® assay, the information below is provided as representative data. Assay was run at ATP Kmapparent and a kinase concentration producing approximately 30-40% of maximal phosphorylation, as discussed in Section 9 and 10 of the Z'-LYTE® protocols. ATP and kinase concentrations should be optimized for each kinase by the actual user. Specific Z'-LYTE® assay protocols and setup information from Invitrogen's own in-house SelectScreen® Custom Profiling Z'-LYTE®-based kinase assay service can be located at the following link: http://www.invitrogen.com/content.cfm?pageid=9866.

1. Prepare initial 100X serial dilution curves in rows A and E of a 384-well plate: Dilute Staurosporine and JAK2 Inhibitor II to a 100X initial concentration in 100% DMSO (100 µM). 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.

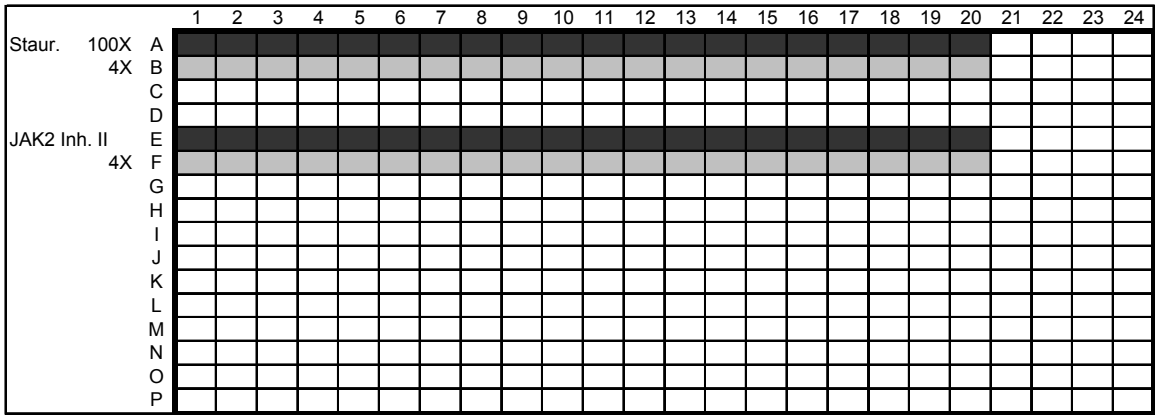



**Figure 1: Schematic of initial compound dilution.** Staurosporine and JAK2 Inhibitor II were titrated from a 100 µM starting concentration in the initial dilution series by preparing a 1:1 dilution curve in DMSO. A secondary dilution to 4X was then prepared in the rows below the initial dilution curve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

2. The 100X serial dilution set is then diluted to a 4X working concentration in Kinase Buffer (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 NBS, 384-well plate (Corning Cat. # 3676), 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 5 µL of the 2X Peptide/Kinase Mixture (2 µM Tyr 06 peptide, 2600 ng/ml JAK2 JH1/JH2 or 1300 ng/ml JAK2 JH1/JH2 V617F, determined experimentally as outlined above) to Columns 1-22. DO NOT ADD TO COLUMN 23 OR 24. Add 5 µL of 2µM substrate alone without kinase to Column 23, rows A-L (0% phosphorylation control) and 5 µl of 2 µM phpsphopeptide control substrate to Column 24, rows A-L (100% phosphorylation control). Add 5 µl kinase buffer alone to the remaining 8 wells (Columns 23 and 24, rows M-P) as a buffer-only reference.

6. Add 2.5 µL of 4X ATP Solution (200 µM) per well to all Columns to start reaction.

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 of the Development Reagent Solution to each well. Use the lot-specific dilutions indicated on your CoA as dilution may vary based upon Z'-LYTE® peptide and Development Reagent A lot.

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

11. Incubate for 60 minutes at room temperature.

12. Read and analyze as directed in the protocol.

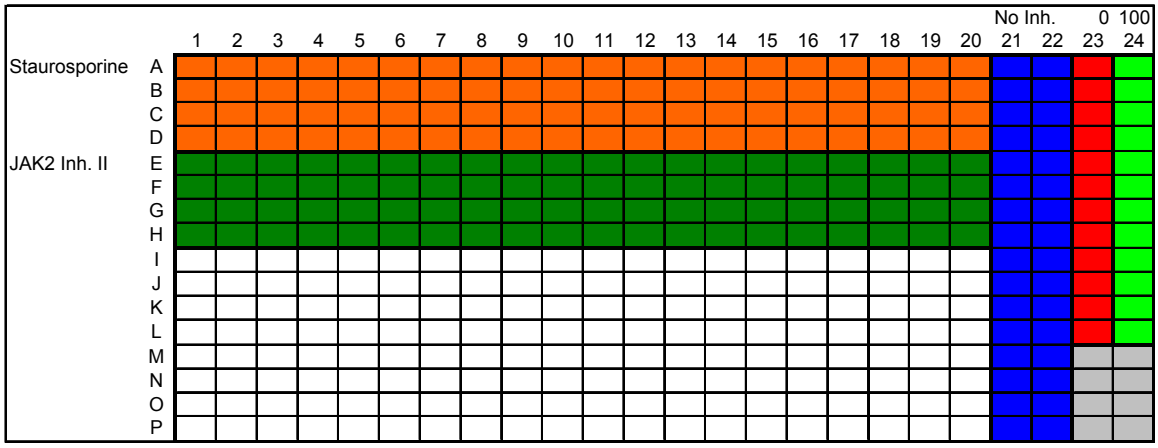



**Figure 2: Assay Plate Schematic.** Compound titrations shown in Columns 1-20, Columns 21 and 22 prepared without any inhibitor as kinase activity controls, Column 23 prepared with no kinase (0% phosphorylation) and Column 24 prepared using phosphopeptide control (100% phosphorylation). Note 8 wells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

## D. Results

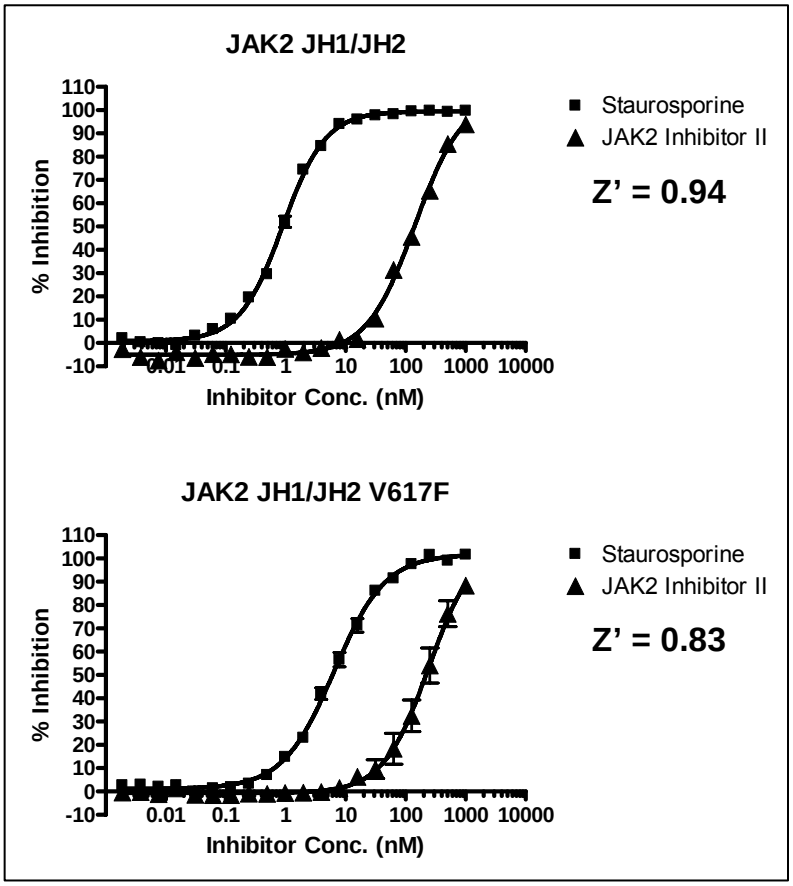



**Figure 1: Z'-LYTE® Kinase Assay.** Z'-LYTE® assay performed using the BMG LABTECH PHERAstar.