# LanthaScreen® Terbium 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 LanthaScreen® Terbium-based TR-FRET Assay using the LanthaScreen® Fluorescein-Poly GT (PV3610) and Tb-PY20 antibody (PV3552) 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.

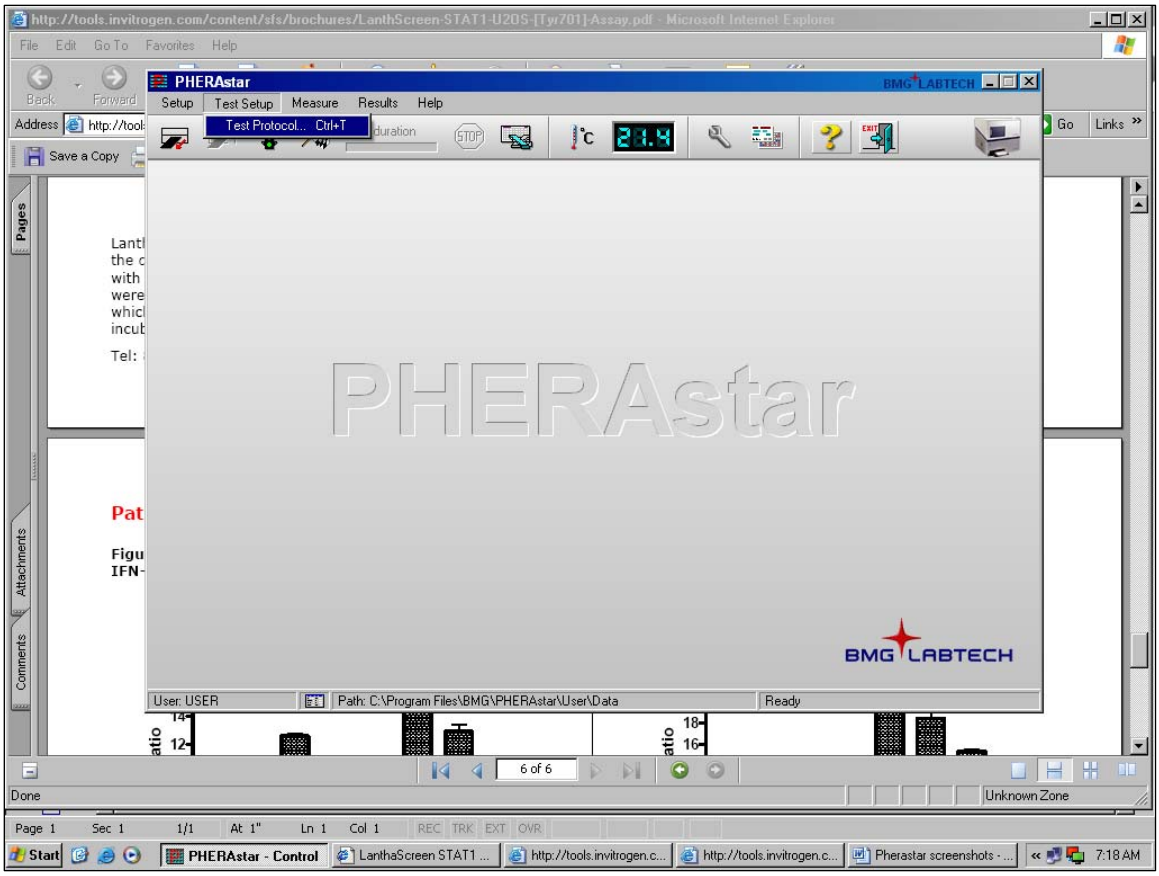
## A. Recommended Optics

| | wavelength (nm) | BMG LABTECH Optic Module |
|---|---|---|
| Excitation | 337 | LanthaScreen |
| Emission 1 | 520 | LanthaScreen |
| Emission 2 | 490 | LanthaScreen |
| Dichroic Mirror | Included | LanthaScreen |

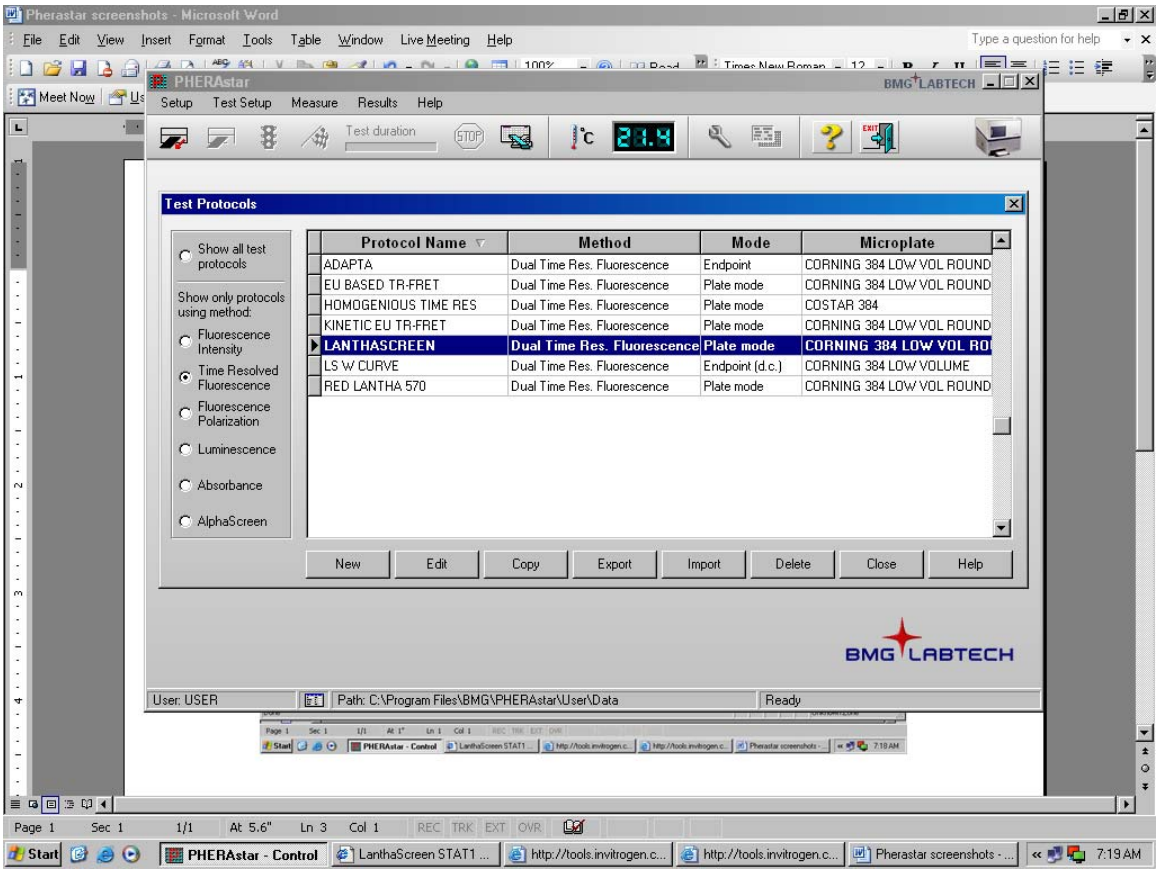
## B. Instrument Setup

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

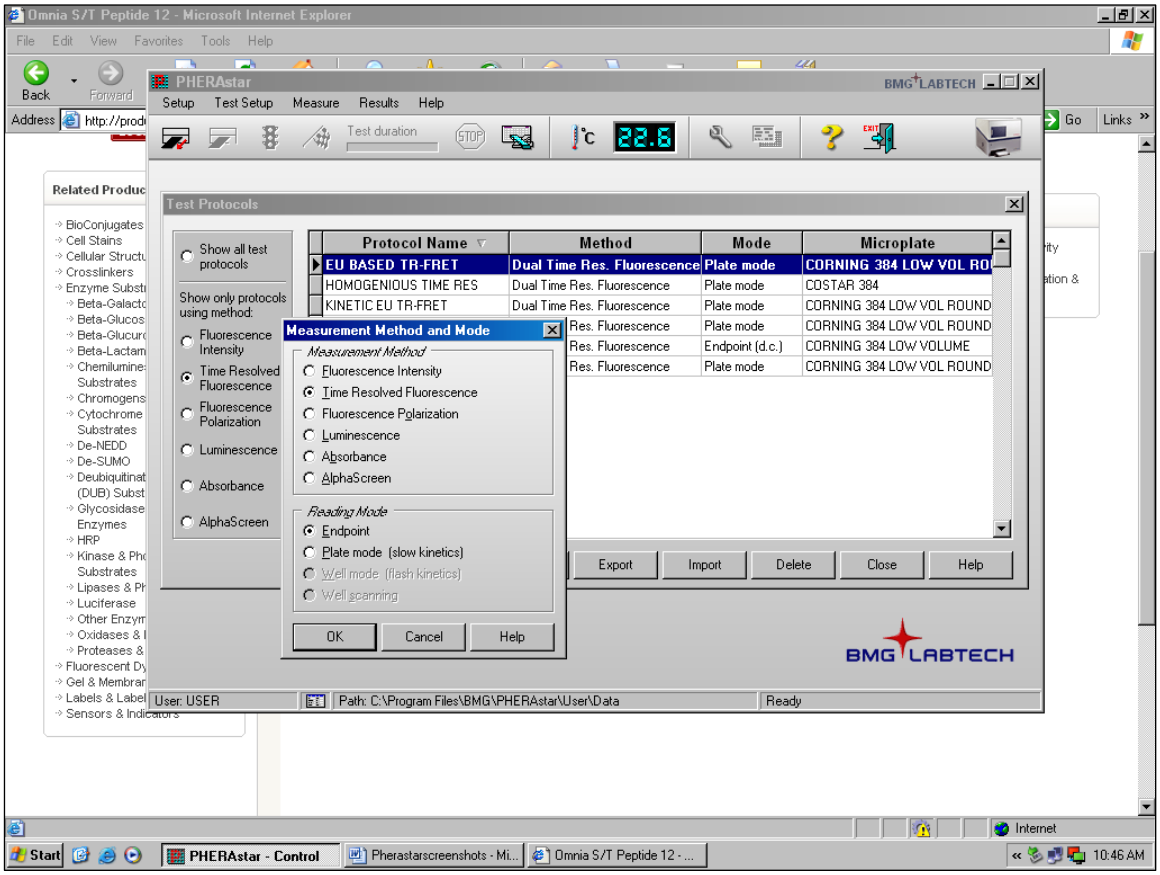
2. When PHERAstar Control software opens, if you do not have a pre-existing protocol for LanthaScreen®, select "Test Protocol" from the Test Setup menu bar at the top of the window.



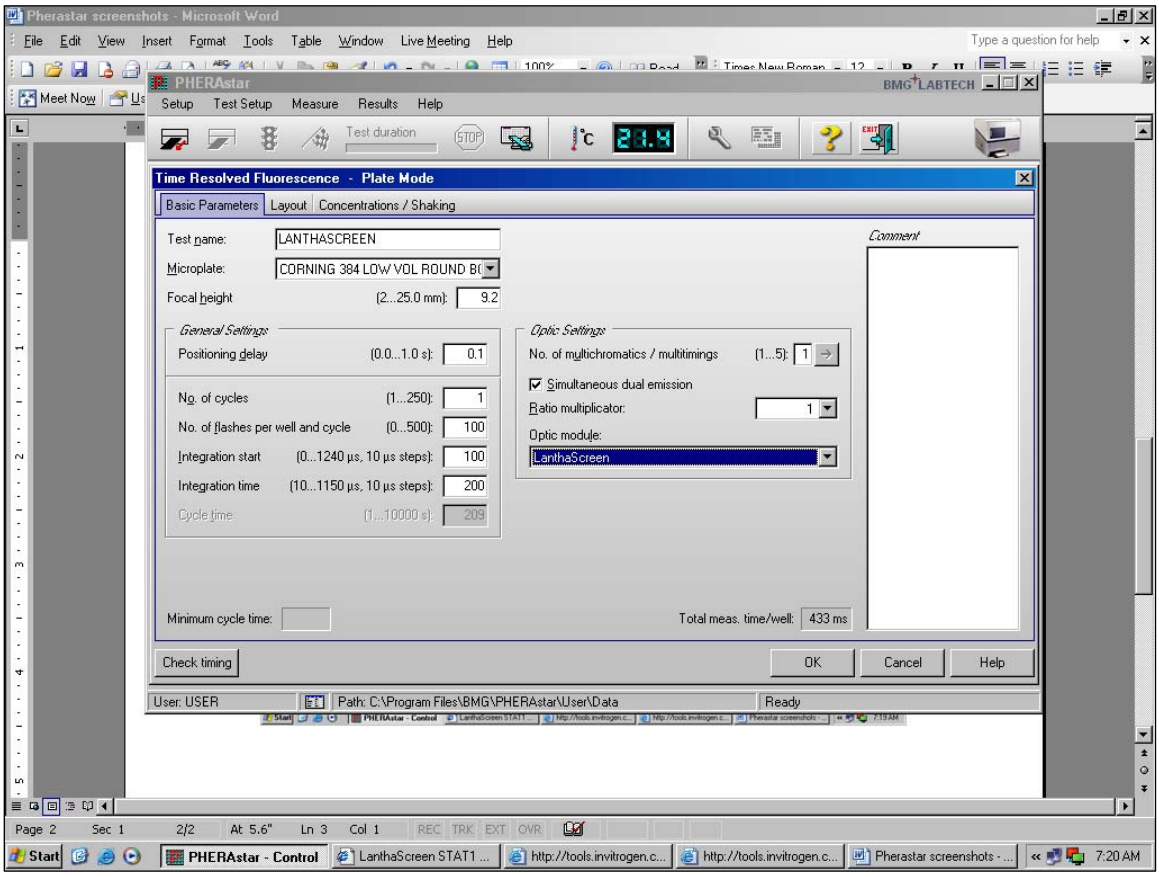
3. At this point, a new screen will open (below). Click on the “Show all test protocols” or "Time Resolved Fluorescence" button on the left side of the screen, then select “New” from the tabs at the bottom.



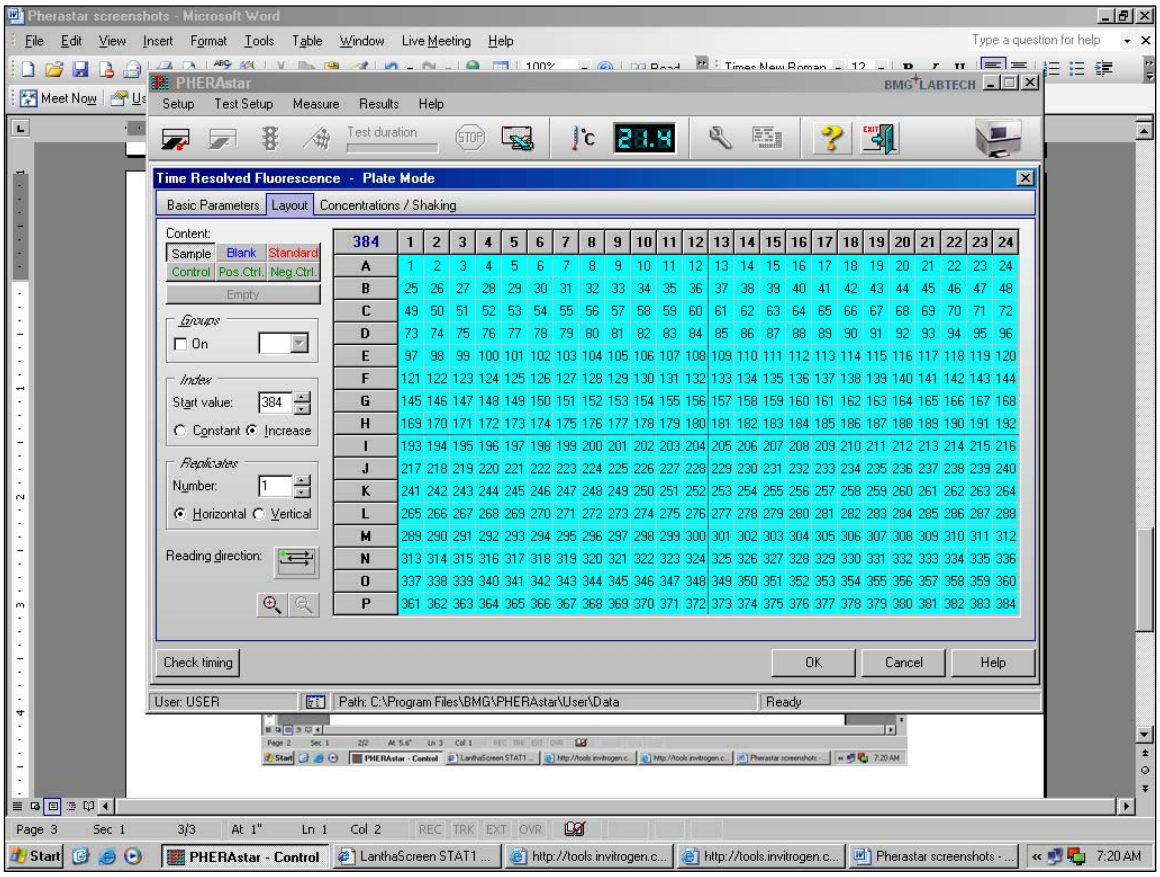
4. A new window will pop up. Select “Time-Resolved Fluorescence” and “Endpoint” and then select “OK”.



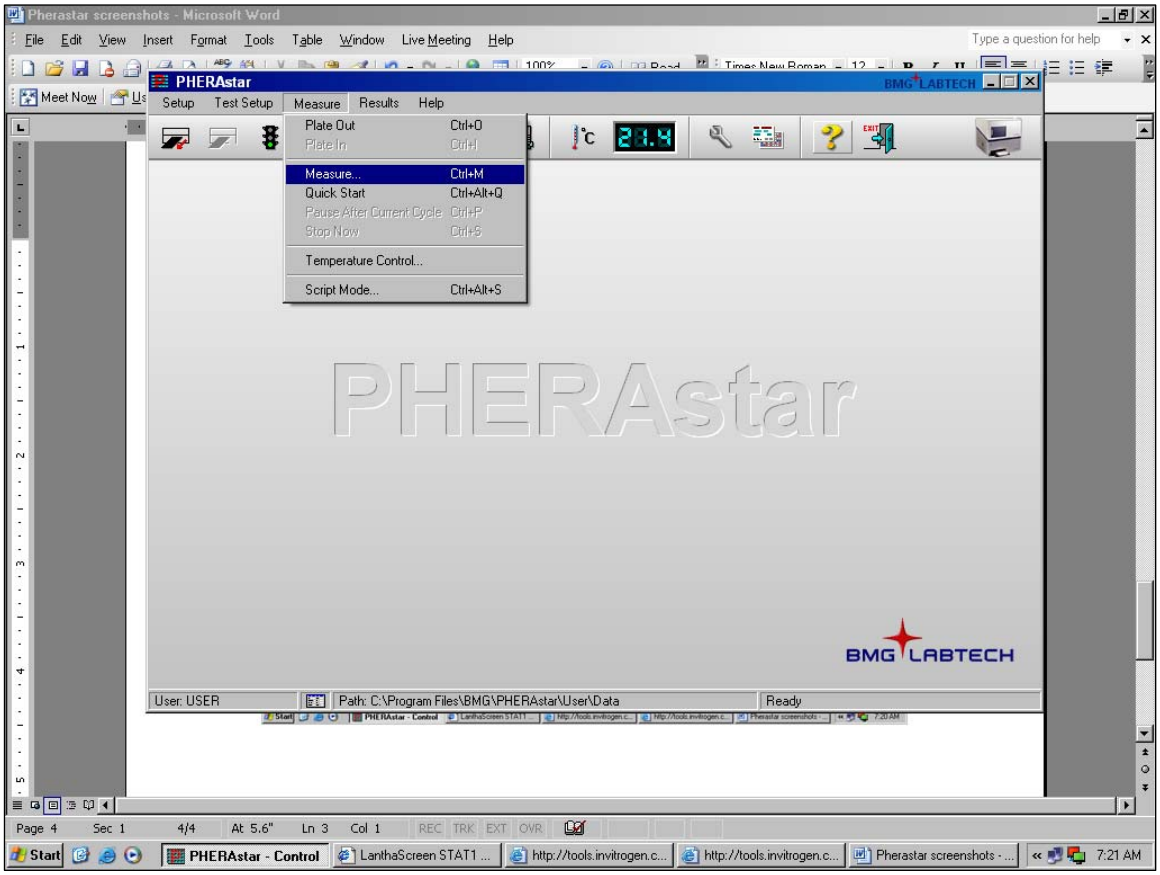
5. A new Protocol window will open automatically. Enter a test name, select plate type, and check the "Simultaneous Dual Emission" box. From the drop-down menu, select your optic module. Because LanthaScreen® is a Time-Resolved FRET assay, set the Integration Start and Integration Time to 100 and 200 µseconds, respectively, as shown. When finished, select the "Layout" tab at the top of the Protocol window.



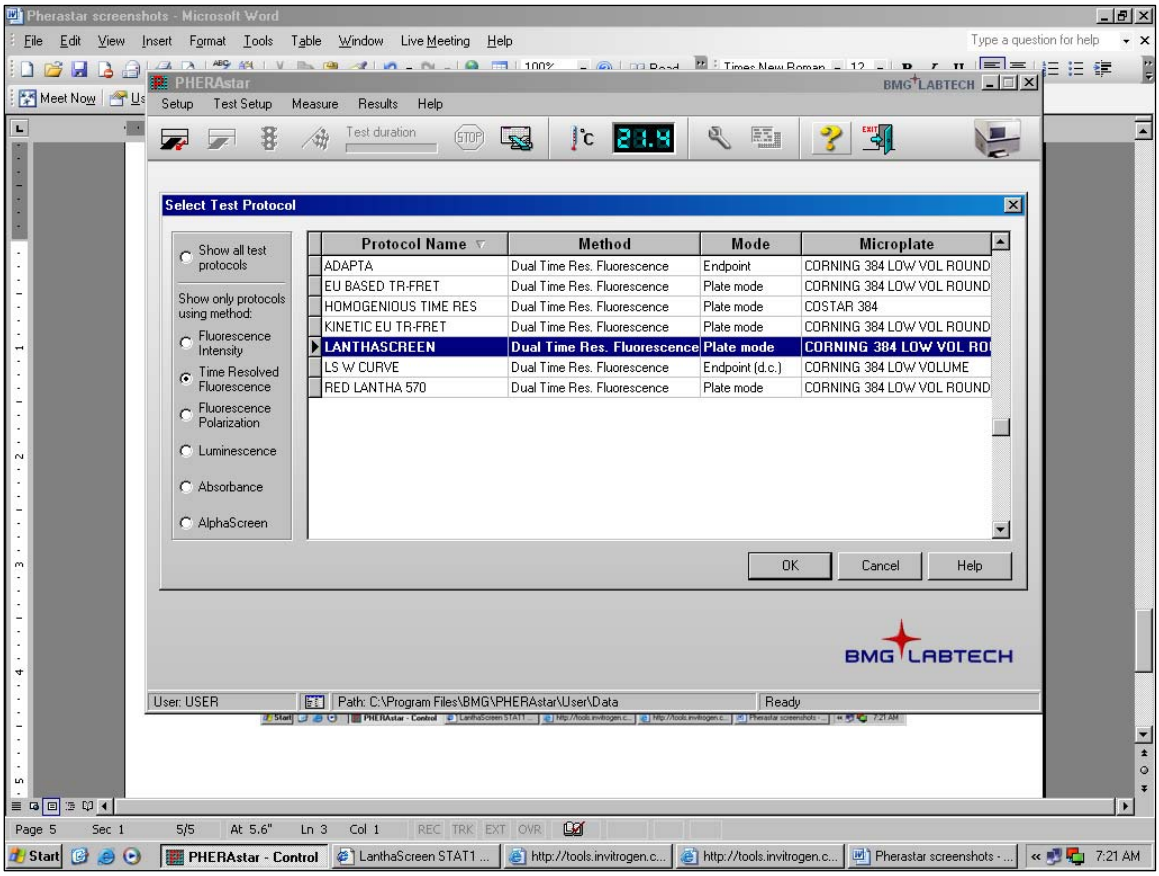
6. Select the wells you wish to read. Note in this step you can select to designate blanks, positive controls, etc. but for this case we marked all wells "Sample" and calculations were performed manually. When finished, select OK.



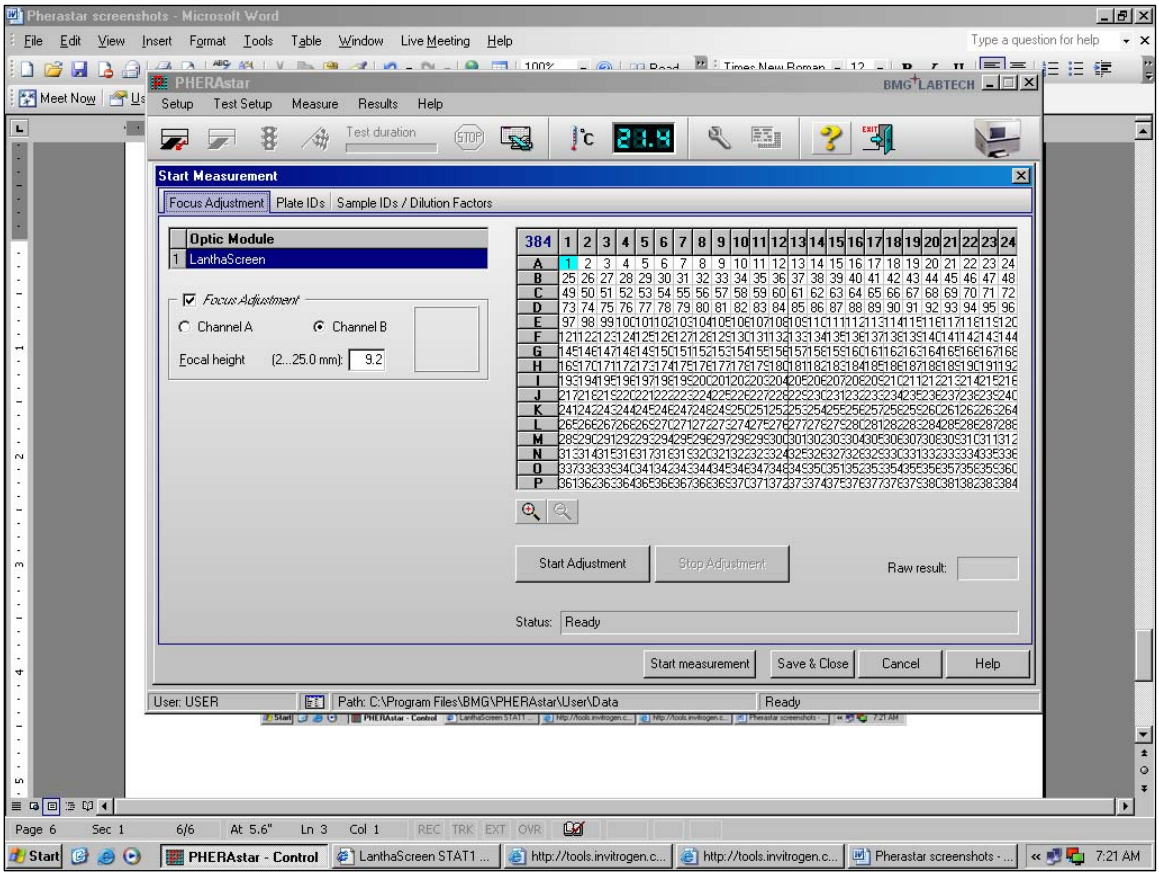
7. You will return to the initial settings window. From the drop-down menus at the top, select "Measure" and "Measure" again.



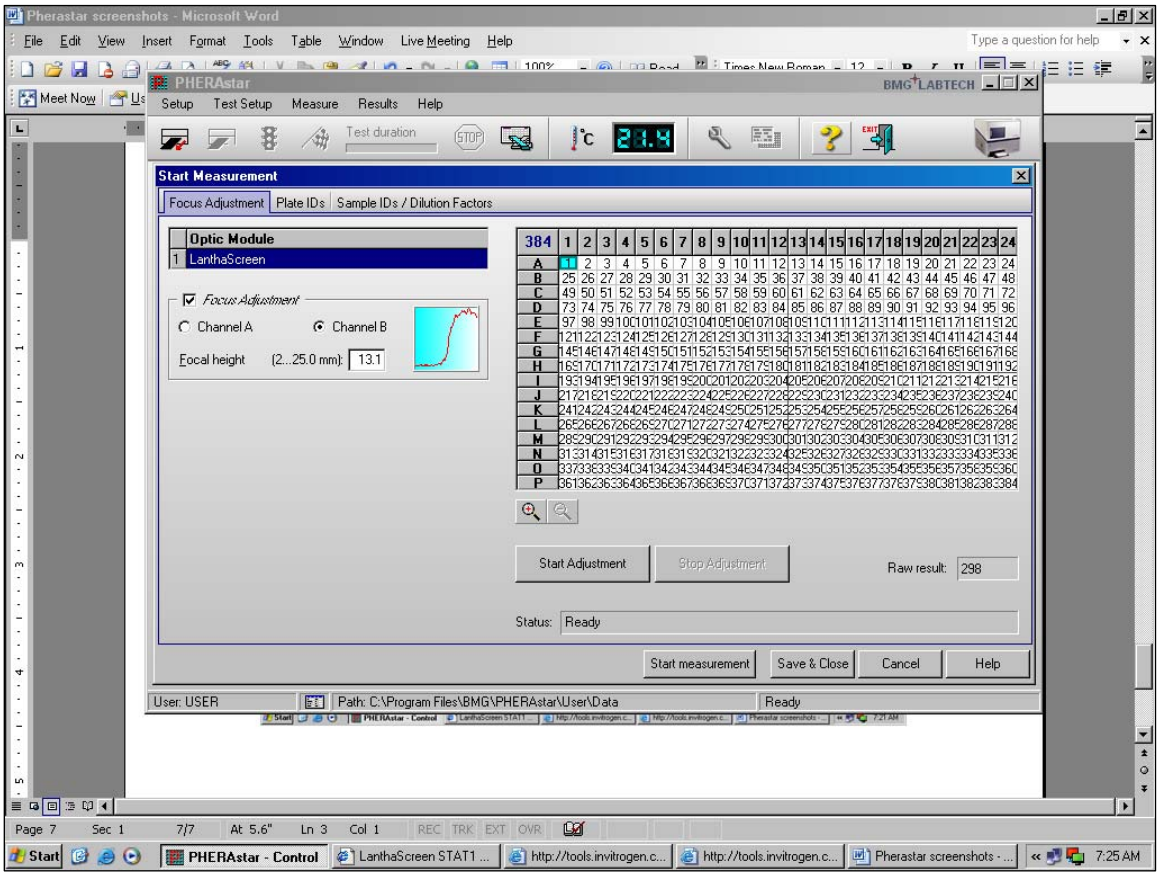
8. A new window will appear allowing you to select which of your test protocols you wish to run. Select the protocol you created for LanthaScreen®, and then press OK.



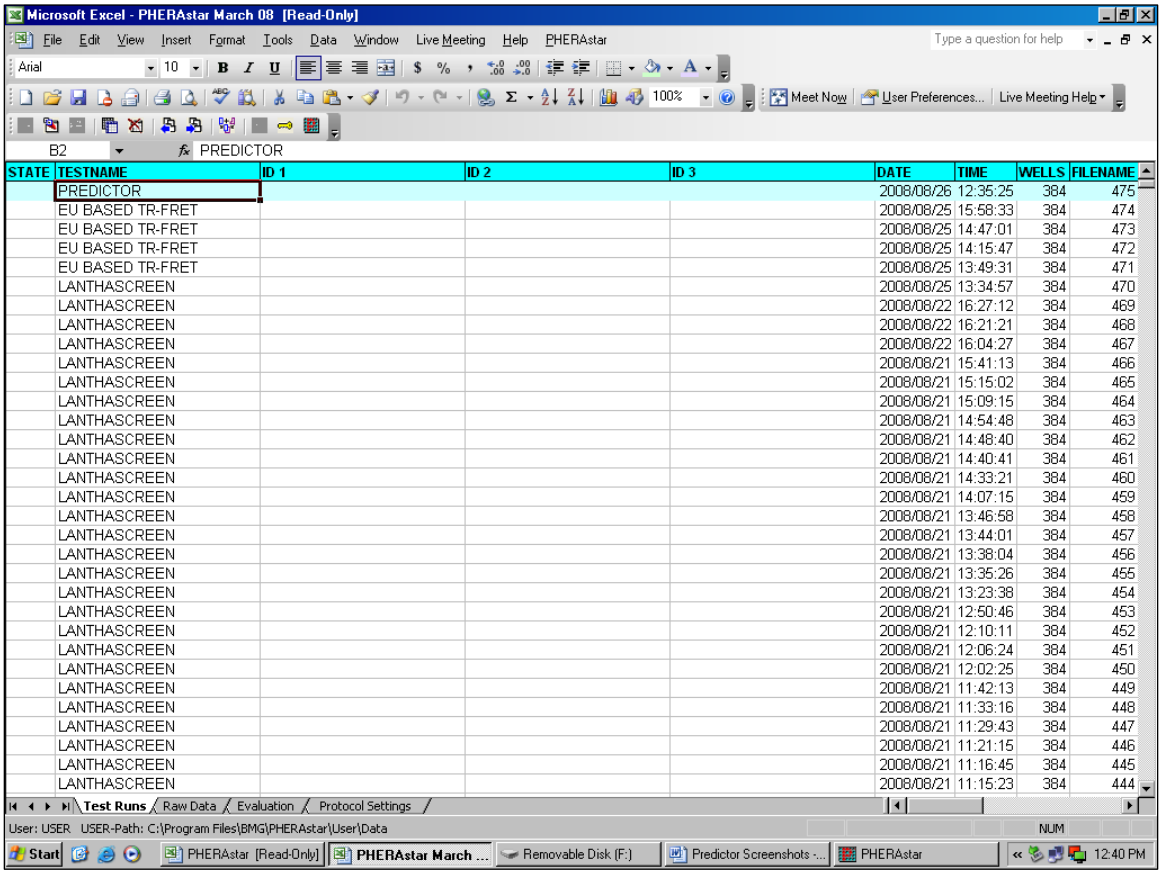
9. A new window will appear. Place your plate in the reader, and select a well to use for adjusting focus by highlighting the well of your choice. When finished, click on the "Start Adjustment" tab.



10. In a moment, the instrument will have calculated the optimal focal height. When 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. LanthaScreen® 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 LanthaScreen® assay, the information below is provided as representative data only and the following section is an explanation of what was done. We recommend all first-time users begin by following the provided assay-specific protocols and/or validation packets, and include proper controls. This assay was run at ATP $Km_{app}$ and a kinase concentration 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, visit www.invtrogen.com/lanthascreenkinase.

1. Prepare initial 100X serial dilution curves in rows A, E, I, and M of a 384-well plate (Figure 1): Dilute Staurosporine, JAK2 Inhibitor 2, JAK3 Inhibitor, and AG-490 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.

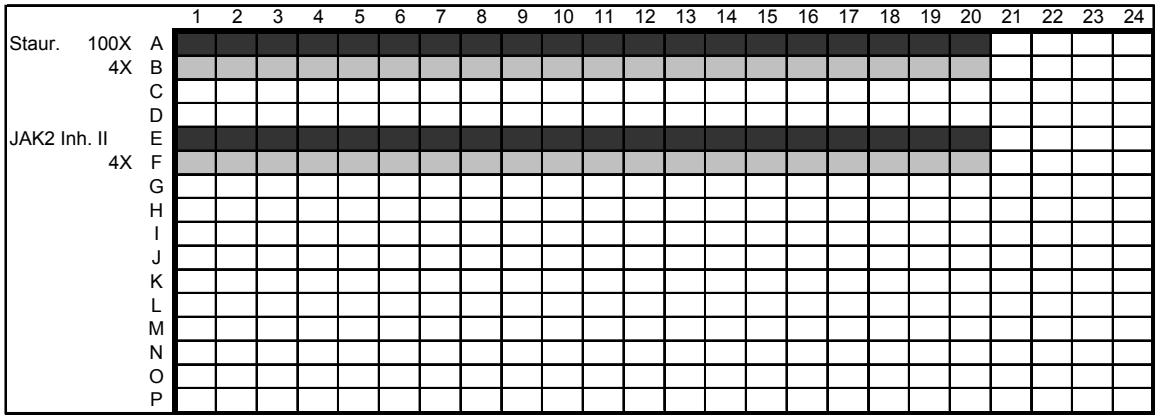



**Figure 1: Schematic of initial compound dilution.** Staurosporine, JAK2 Inhibitor II, JAK3 Inhibitor, and AG-490 were all titrated from a 100 µM starting concentration in the initial dilution series by preparing a 1:1 dilution curve in DMSO. A secondary dilution to 4X was then prepared in the rows 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 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 (800 nM Fluorescein-Poly GT peptide, PV3610, 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 5 µL of 800 nM substrate alone without kinase to Column 23, rows A-L (0% phosphorylation control) and 5 µl of 800 nM phosphopeptide control substrate to Column 24, rows A-L (100% phosphorylation control). Add 5µl 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 (20 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 10 µl per well of 20 mM EDTA, 2 nM TB-PY20 antibody (PV3552) mix diluted in TR-FRET Dilution Buffer (PV3574) per well to stop kinase reaction. Do not add to Rows M-P,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.

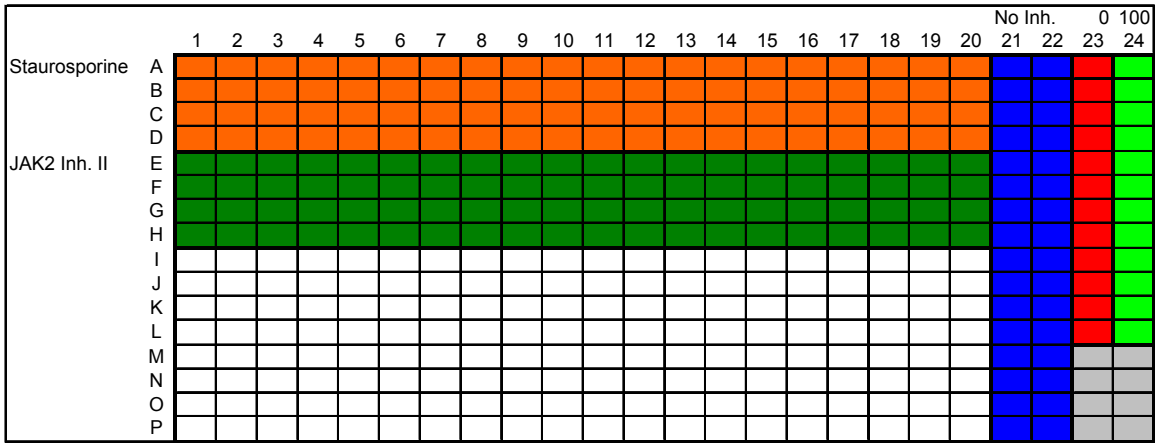



**Figure 2: Assay Plate Schematic.** Compound titrations shown in Columns 1-20, Columns 21 and 22 prepared without any inhibitor as kinase activity controls, Column 23 prepared with no kinase (0% phosphorylation) and Column 24 prepared using phosphopeptide control (100% phosphorylation). Note 8 wells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

## D. Results:

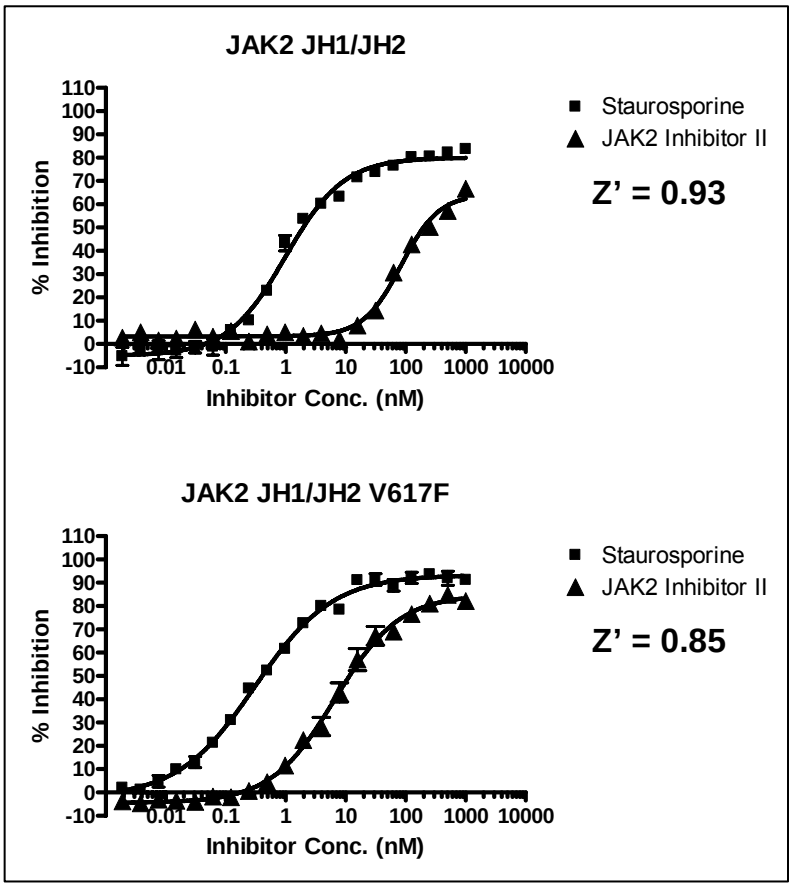



**Figure 3: LanthaScreen® Assay.** LanthaScreen® assay performed on the BMG LABTECH PHERAstar.