PhosphoSens® Assay Setup Guide on the

BMG LABTECH PHERAstar/PHERAstarPlus /PHERAstar FS/PHERAstar FSX

Microplate Readers

The following document is intended to demonstrate the setup of this instrument.

For more detailed instrument information and technical support of BMG LABTECH instruments or software, please contact BMG LABTECH at 1-877-264-5227 or www.bmglabtech.com.

For more detailed assay information and technical support of PhosphoSens assays please contact us at support@assayquant.com.

Recommended Optics

|	wavelength (nm)	BMG LABTECH Optic Module
Excitation	360 (or similar)	*contact BMG LABTECH
Emission 1	485 (or similar)	*contact BMG LABTECH
Dichroic Mirror		*contact BMG LABTECH

Instrument Setup

1. Make certain plate reader is turned on, and open up PHERAstar FSX Control software on computer. Insert plate into plate reader.
2. When Control software opens, if you do not have a pre-existing protocol for PhosphoSens, select "Test Protocol" from the Test Setup menu bar at the top of the window.
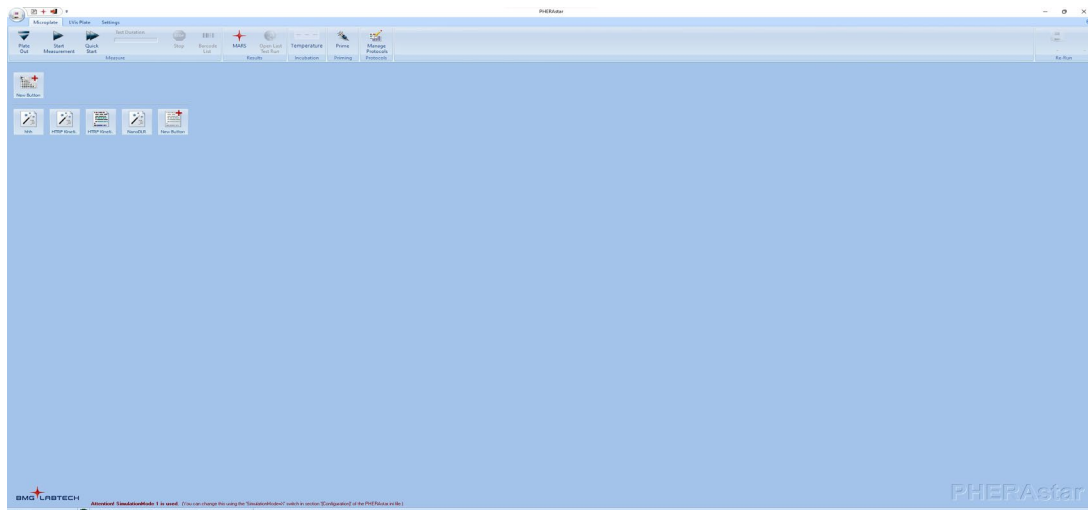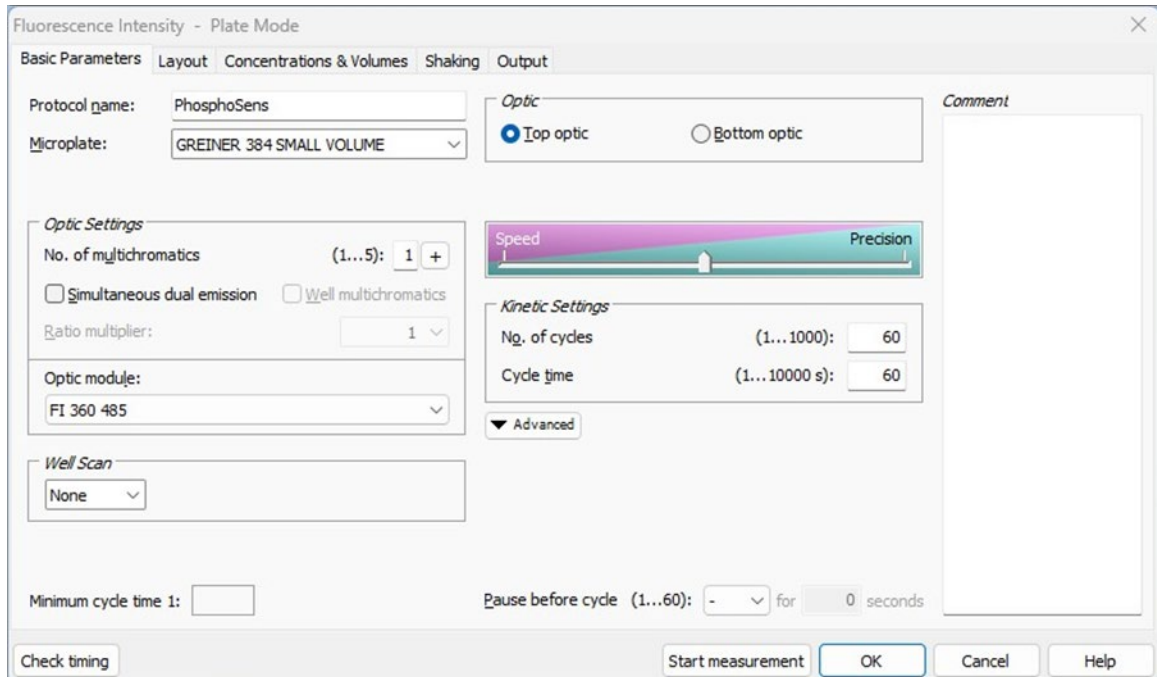
3. At this point, a new screen will open (below). Click on the "Show all test protocols" or "Fluorescence Intensity" button on the left side of the screen, then select "New" from the tabs at the bottom.
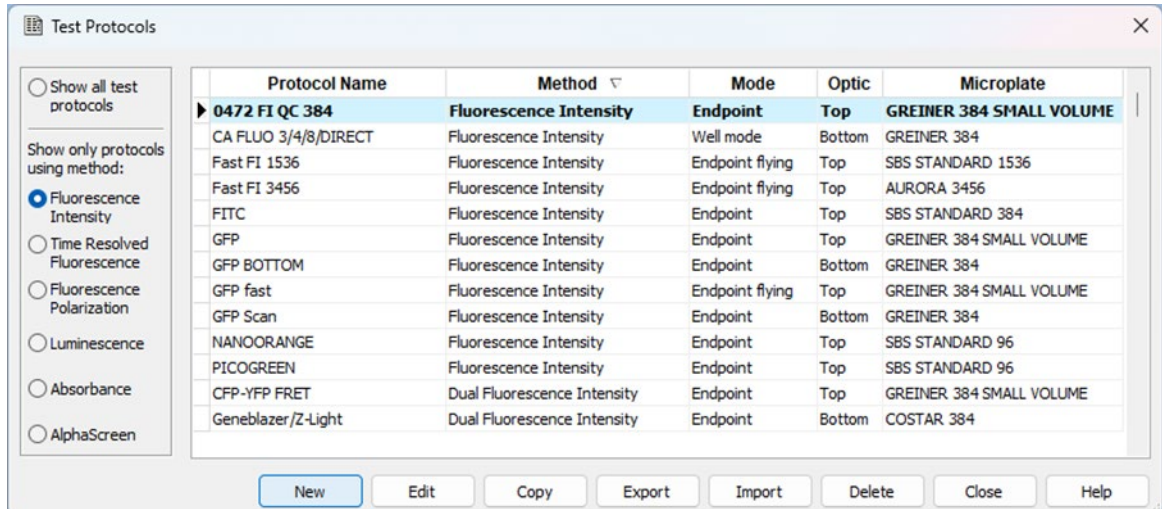
4. A new window will pop up. Select "Fluorescence Intensity" and "Plate mode (slow kinetics)" and then select "OK".
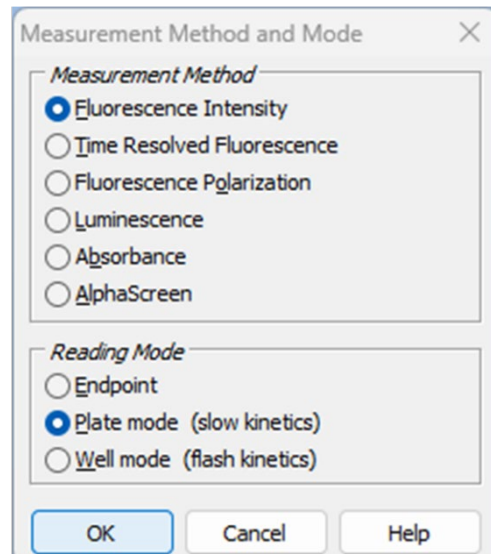
5. A new Protocol window will open automatically. Enter a test name and select plate type. From the drop-down menu, select your optic module. Because Omnia is a kinetic assay, enter the desired number of cycles and the desired cycle duration. In this case we set up a 60-minute assay with one-minute read intervals. When finished, select the "Layout" tab at the top of the Protocol window.

6. Select the wells you wish to read. When finished, select OK.

7. You will return to the initial settings window. Select 'Start Measurement'
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 PhosphoSens, and then press OK.
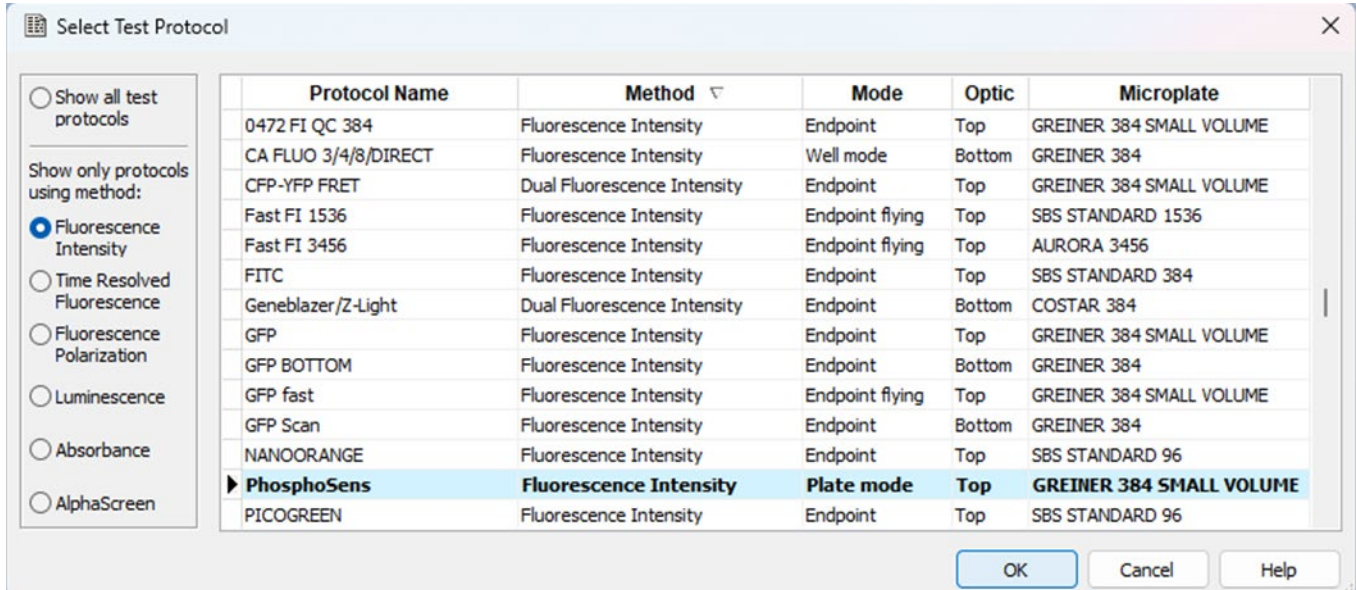
9. A new window will appear. Place your plate in the reader, and select a well to use for adjusting gain and focus by highlighting the well of your choice. The gain or sensitivity can be adjusted at this point, in this case a positive control (phosphopeptide) should be used to avoid going off scale during the assay. When finished, click on the "Start Adjustment" tab.
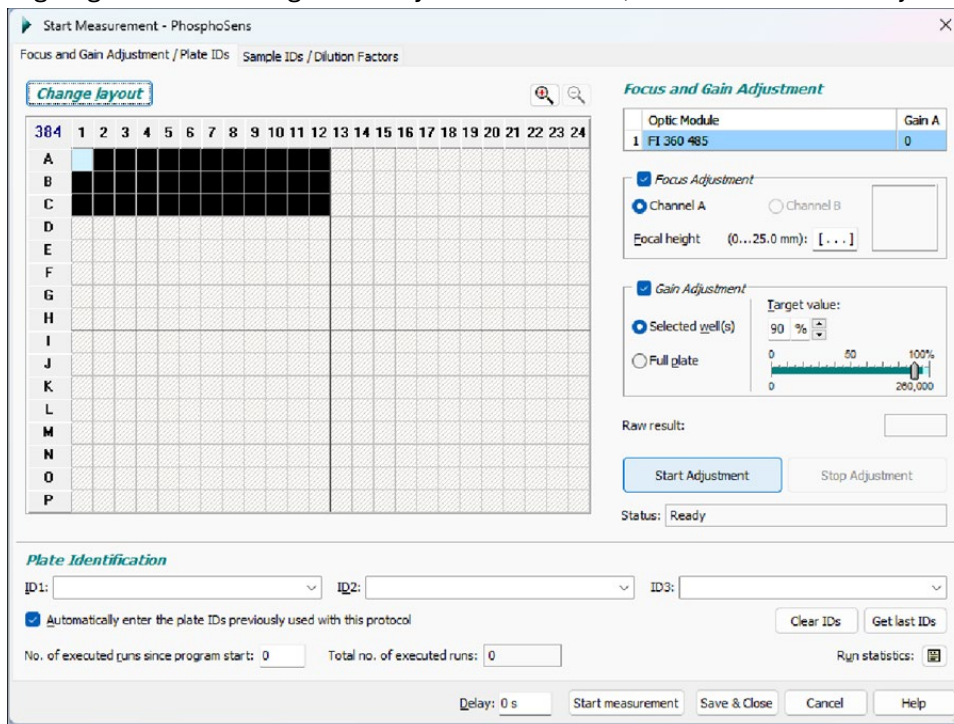
10. In a moment, the instrument will have calculated its optimal focal height and the gain adjustments necessary. When finished, click on the "Start Measurement" tab to read.

11. When PHERAstar FSX is done reading, you can collect your data by clicking "Open Last Test Run" on the toolbar at the top of the window. This will automatically redirect you to BMG's data analysis software MARS which collects run data. Subsequently the last test will open to a view of the data in a plate layout format.