

### 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 <a href="https://www.bmglabtech.com">www.bmglabtech.com</a>.

For more detailed assay information and technical support of PhosphoSens assays please contact us at <a href="mailto:support@assayquant.com">support@assayquant.com</a>.

### **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.

O Show all test	Protocol Name	Method V	Mode	Optic	Microplate					
protocols	0472 FI QC 384	Fluorescence Intensity	Endpoint	Тор	GREINER 384 SMALL VOLUM					
Channelly and have be	CA FLUO 3/4/8/DIRECT	Fluorescence Intensity	Well mode	Bottom	GREINER 384					
using method:	Fast FI 1536	Fluorescence Intensity	Endpoint flying	Тор	SBS STANDARD 1536					
- Elugranconco	Fast FI 3456	Fluorescence Intensity	Endpoint flying	Тор	AURORA 3456					
Intensity	FITC	Fluorescence Intensity	Endpoint	Тор	SBS STANDARD 384					
Time Resolved	GFP	Fluorescence Intensity	Endpoint	Тор	GREINER 384 SMALL VOLUME					
Fluorescence	GFP BOTTOM	Fluorescence Intensity	Endpoint	Bottom	GREINER 384					
○ Fluorescence	GFP fast	Fluorescence Intensity	Endpoint flying	Тор	GREINER 384 SMALL VOLUME					
Polarization	GFP Scan	Fluorescence Intensity	Endpoint	Bottom	GREINER 384					
Luminescence	NANOORANGE	Fluorescence Intensity	Endpoint	Тор	SBS STANDARD 96					
	PICOGREEN	Fluorescence Intensity Endpoint Top SBS STANDAR								
Absorbance	CFP-YFP FRET	Dual Fluorescence Intensity	Тор	GREINER 384 SMALL VOLUME						
AlphaScreen	Geneblazer/Z-Light	Dual Fluorescence Intensity	Endpoint	Bottom	COSTAR 384					

4. A new window will pop up. Select "Fluorescence Intensity" and "Plate mode (slow kinetics)" and then select "OK".

Measurement Method and Mode $$
Measurement Method
Eluorescence Intensity
○ <u>T</u> ime Resolved Fluorescence
O Fluorescence Polarization
○ <u>L</u> uminescence
○ A <u>b</u> sorbance
○ <u>A</u> lphaScreen
Reading Mode
○ Endpoint
<ul> <li>Plate mode (slow kinetics)</li> </ul>
○ <u>W</u> ell mode (flash kinetics)
OK Cancel Help



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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.

Protocol <u>n</u> ame:	PhosphoSens	Optic		Comment	
Microplate:	GREINER 384 SMALL VOLUME	O Top optic	○ <u>B</u> ottom optic		
- <i>Optic Settings</i> - No. of m <u>u</u> ltichro	matics (15): 1 +	Speed	Preds	ion	
Simultaneou	s dual emission <u>W</u> ell multichromatics	<i>Kinetic Settings</i> N <u>o</u> . of cycles	(11000):	0	
Optic module: FI 360 485	~]	Cycle time	(110000 s):	0	
Well Scan		• Advanced			
Minimum cvcle tim	e 1:	Pause before cycle (16	0): - V for 0 sec	onds	

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

ontent:	384	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	2
Sample Blank Standard	A	1	2	3	4	5	6	7	8	9	10	11	12												-
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- 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.

O Show all test	Protocol Name	Method  \(\nabla \)	Mode	Optic	Microplate				
protocols	0472 FI QC 384	Fluorescence Intensity	Endpoint	Тор	GREINER 384 SMALL VOLUME				
Show only protocols using method: CA	CA FLUO 3/4/8/DIRECT	Fluorescence Intensity	Well mode	Bottom	GREINER 384				
	CFP-YFP FRET	Dual Fluorescence Intensity	Endpoint	Тор	GREINER 384 SMALL VOLUME				
Eluorescence	Fast FI 1536	Тор	SBS STANDARD 1536						
Intensity	Fast FI 3456	Fluorescence Intensity	Endpoint flying	Тор	AURORA 3456				
Time Resolved	FITC	Fluorescence Intensity	Endpoint	Тор	SBS STANDARD 384				
Fluorescence Geneblazer/Z-Light		Dual Fluorescence Intensity	OSTAR 384						
O Fluorescence Polarization	GFP	Fluorescence Intensity	Endpoint	Тор	GREINER 384 SMALL VOLUME				
	GFP BOTTOM	Fluorescence Intensity	Endpoint	Bottom	GREINER 384				
Luminescence	GFP fast	Fluorescence Intensity	Endpoint flying	Тор	GREINER 384 SMALL VOLUME				
	GFP Scan	Fluorescence Intensity	Endpoint	Bottom	GREINER 384				
Absorbance	NANOORANGE	Fluorescence Intensity	Endpoint	Тор	SBS STANDARD 96				
0.000	PhosphoSens	Fluorescence Intensity	Plate mode	Тор	GREINER 384 SMALL VOLUME				
AlphaScreen	PICOGREEN	Fluorescence Intensity	Endpoint	Тор	SBS STANDARD 96				

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.