LanthaScreen® Terbium Assay Setup Guide on the Molecular Devices FlexStation® Microplate Reader

NOTE: The Molecular Devices FlexStation® Microplate Reader was tested for compatibility with Invitrogen's LanthaScreen® Terbium-based TR-FRET Assay. The following document is intended to demonstrate setup of this instrument. 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 Molecular Devices instruments or software, please call 1-800-635-5577 or by e-mail at info@moldev.com.

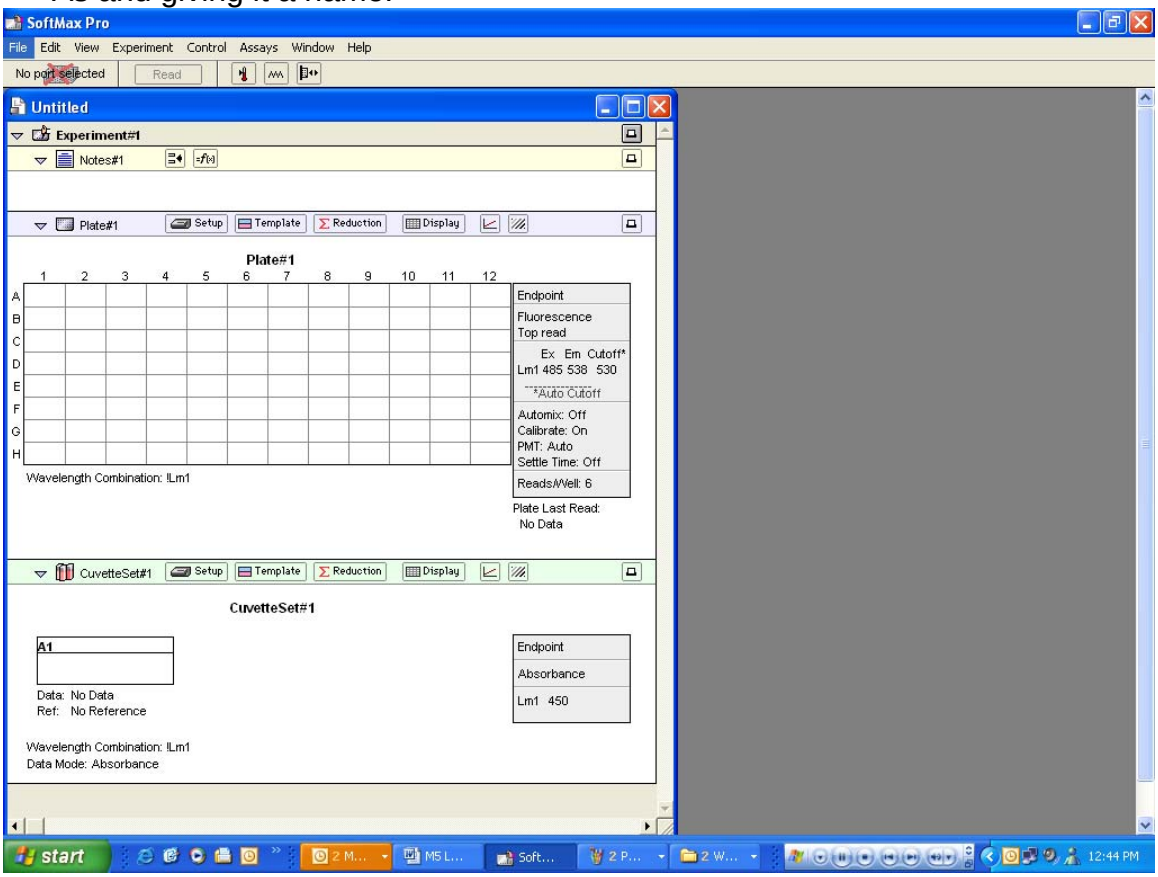
A. Recommended Optics

	wavelength (nm)	diameter (mm)
Excitation	332/12	monochromator
Emission 1	488/12	monochromator
Emission 2	518/12	monochromator
Dichroic Mirror	420	

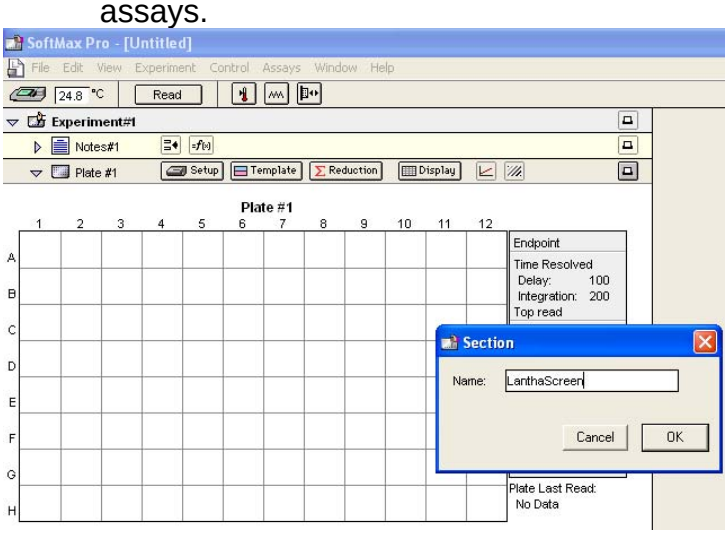
B. Instrument Setup

1. Open the SoftMax Pro software and click on File/New.

The following screen shows up. You can save this new "Protocol" by clicking on File/Save As and giving it a name.

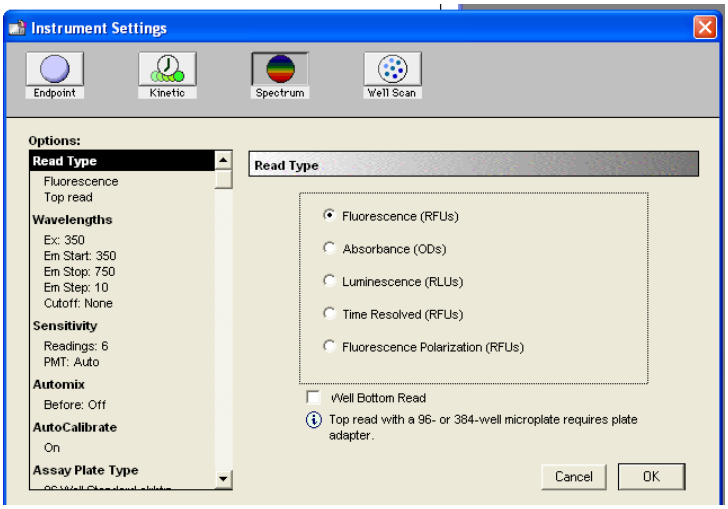


2. You can double click on Plate #1 and rename this section as shown in the example below. This is not necessary but could be helpful in order to keep track of different assays.

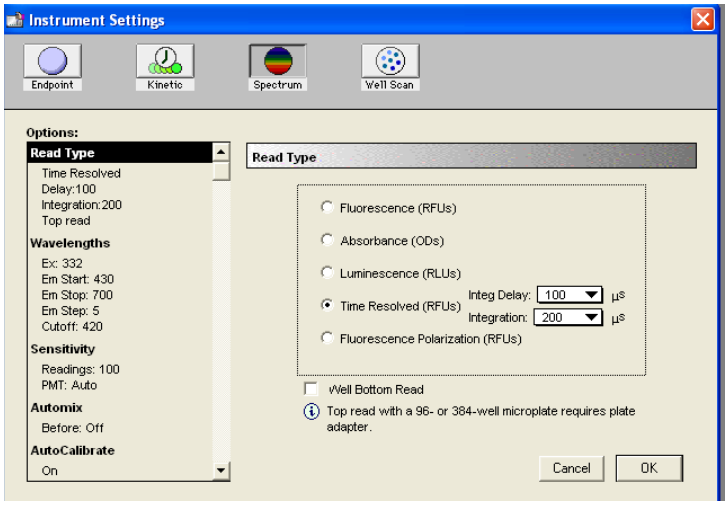


3. We find it convenient to run a wavelength spectrum from a well that contains only the Tb labeled antibody. This gives you visual proof that the wavelengths you are using are the optimal. Skip to step # 11 if you do not want to do a Spectrum analysis.

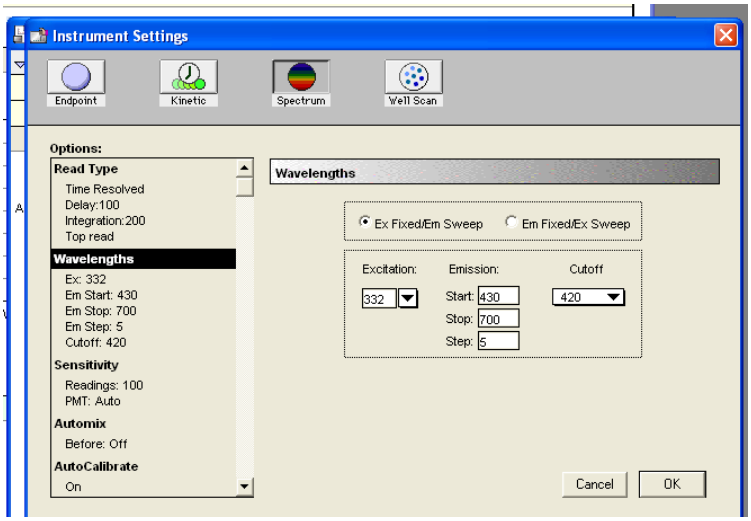
   Click on the Set Up icon right next to LanthaScreen® and the following screen appears:



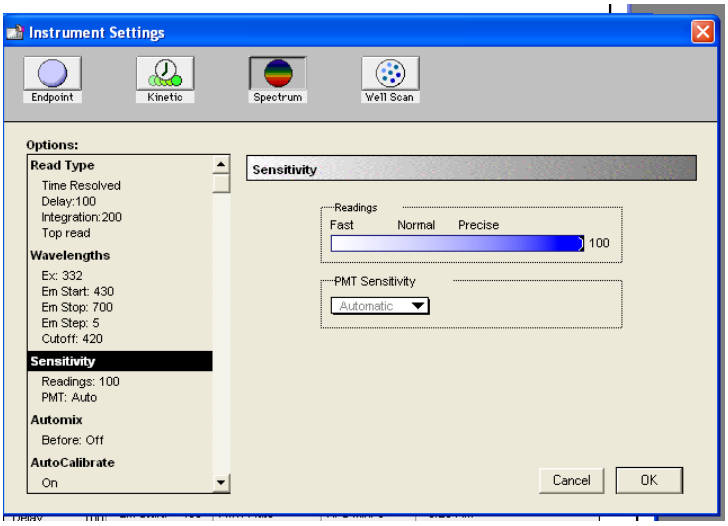
4. Check Time Resolved and enter the values shown below (Integ Delay 100 µs, Integration = 200 µs)



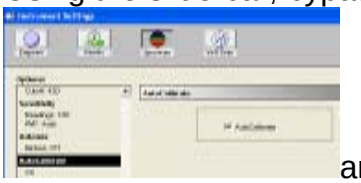
5. Click on Wavelengths, choose Ex Fixed/Ems Sweep and enter the information shown below (Excitation 332, Emission Start 430, Stop 700, Step 5, Cutoff 420).



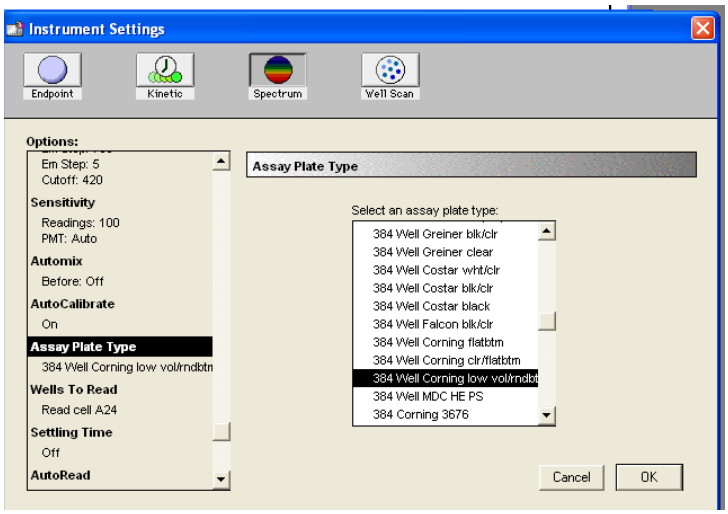
6. Click on Sensitivity and move the slider all the way to the right for optimal sensitivity (=100). Note that PMT Sensitivity = Automatic is the only choice for Spectrum.



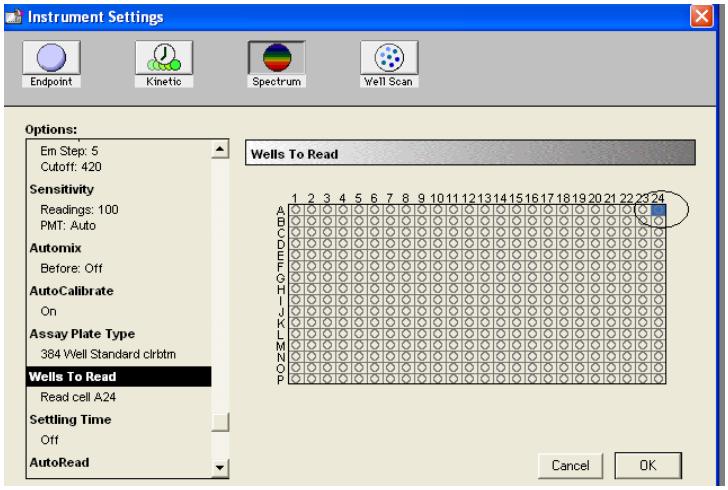
7. Using the slide bar, bypass Automix, make sure AutoCalibrate is chosen



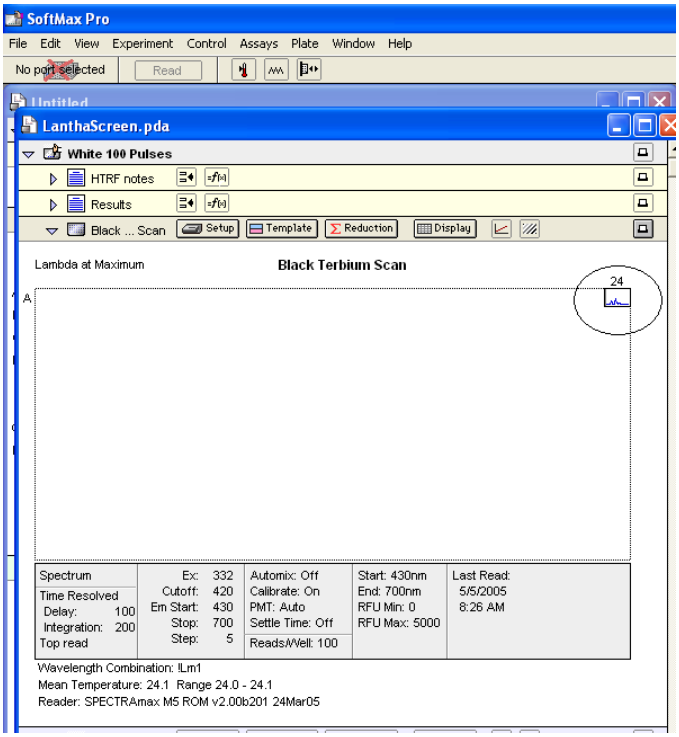
and double click on Assay Plate type. Choose the appropriate plate from the drop down menu.



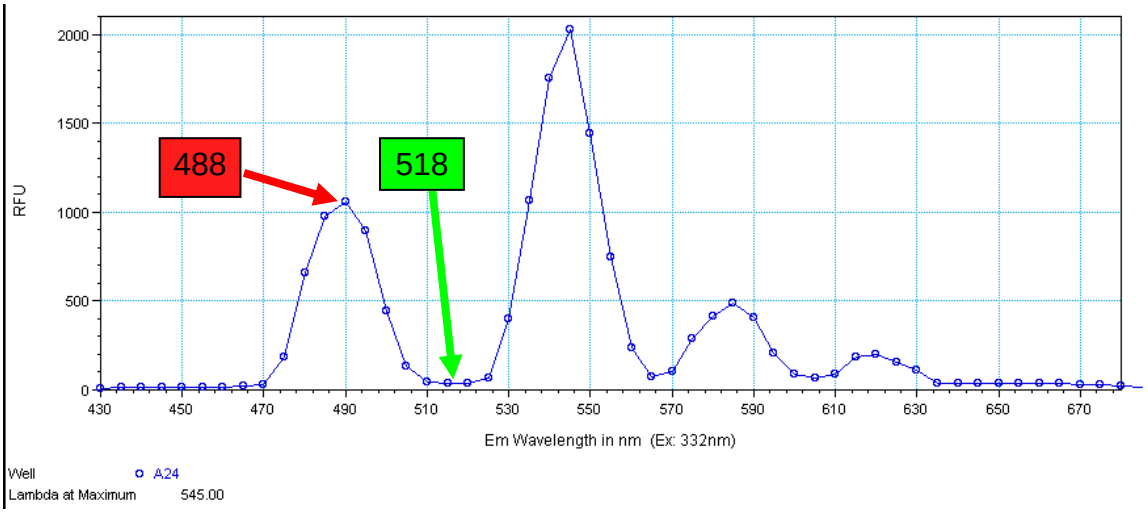
8. Click on Wells To Read and choose one well containing only the Tb labeled Ab. In the example below, we have chosen A24.



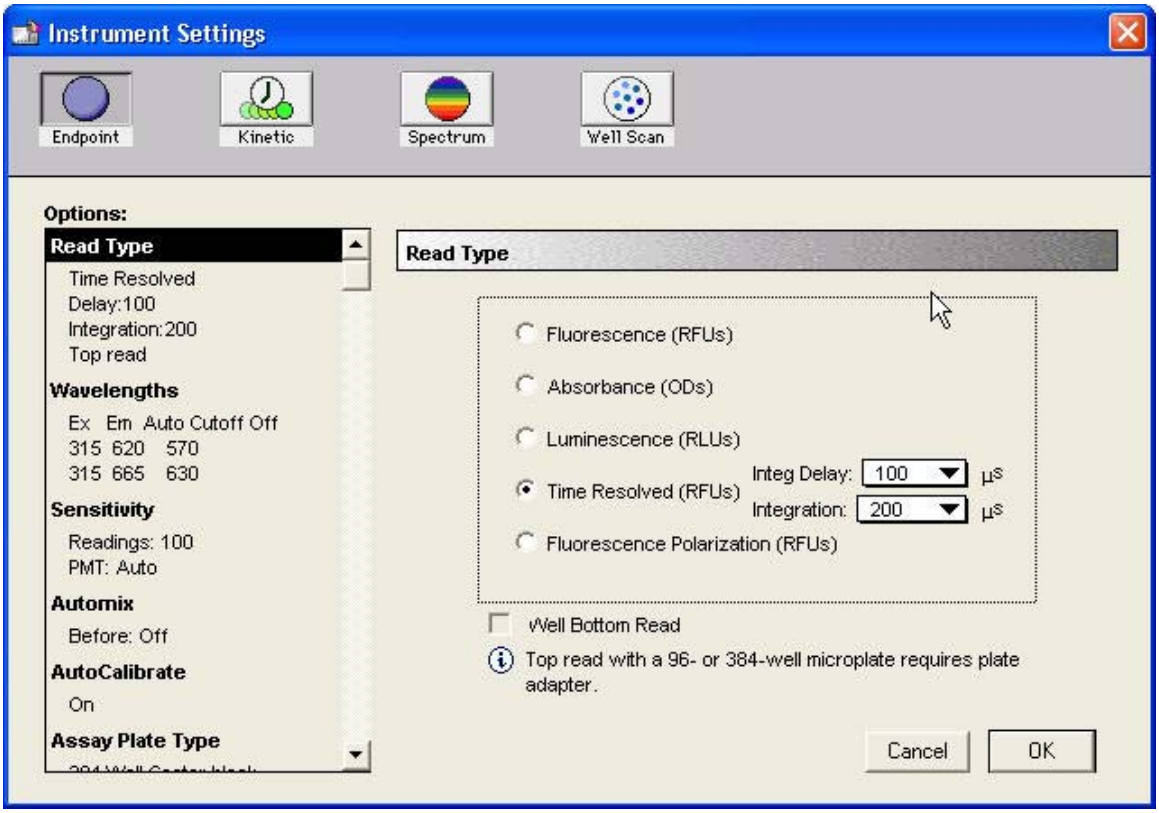
9. Click on OK and then Read. After the read, the following screen will appear:



10. Double click on the well A24 and the Spectrum Graph show up. In our test, this is the graph we generated. Note that we chose 488 nm for the donor reference and 518 for the acceptor.

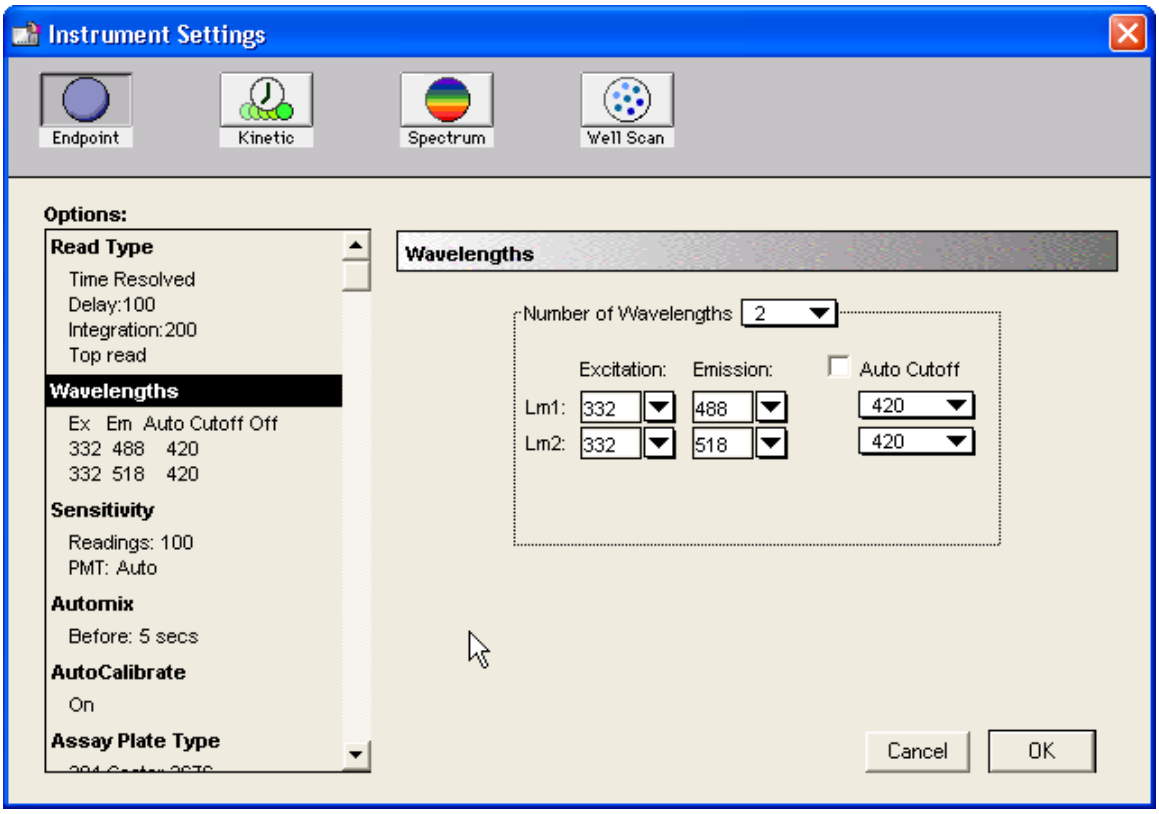


11. Click on the Set Up icon right next to LanthaScreen® and the following screen appears:
    a. Click on Endpoint, followed by Read Type (under Options).
    b. Click on Time Resolved and enter 100 for Integ Delay and 200 for Integration.

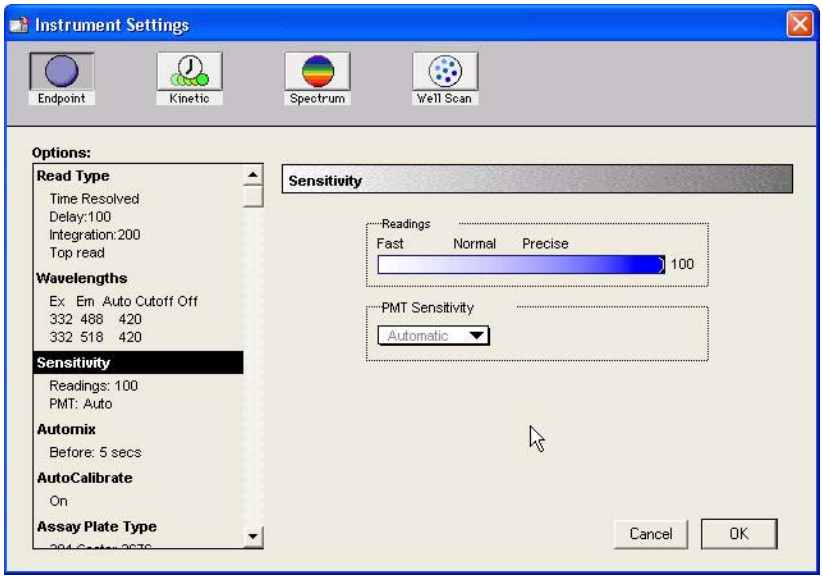


12. Click on Wavelengths and enter the following information: Number of Wavelengths = 2; Lm1 Excitation = 332, Emission = 488; Lm2 Excitation = 332, Emission = 518; Cutoff for both Lm1 and Lm2 = 420 (unclick Auto Cutoff to enable the cutoff choices). If the wavelength or cutoff is not one of the default choices, simply manually enter it.

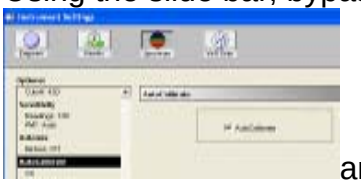
Note there is no choice for bandpass: it is built in at 12nm.



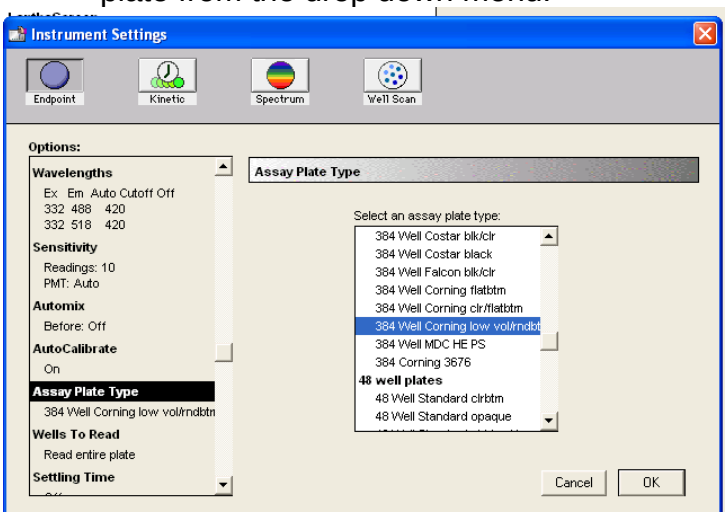
13. Click on Sensitivity and move the slider until it reads 100, all the way to the right. This is the most sensitive setting (100 reads/well) and therefore takes the most time (~15 minutes/384 well plate). We have tried the same plate at 100 reads and at 10 reads with very little difference in data quality but great savings in time (<3 minutes at 10 reads/ well). See Figure 1 and Table 1 below.



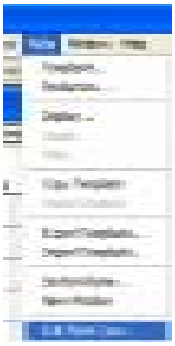
14a. Using the slide bar, bypass Automix, make sure AutoCalibrate is chosen



and double click on Assay Plate type. Choose the appropriate plate from the drop down menu.

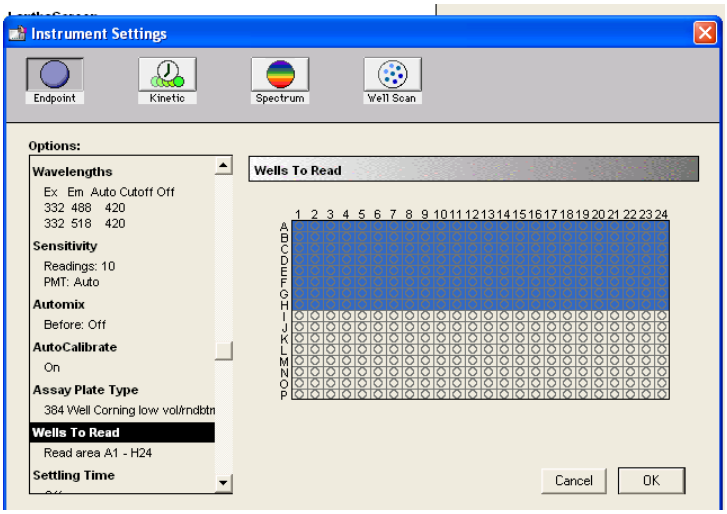


14b. If you need to enter plate information, select Edit Plate Type from the Plate drop down menu.

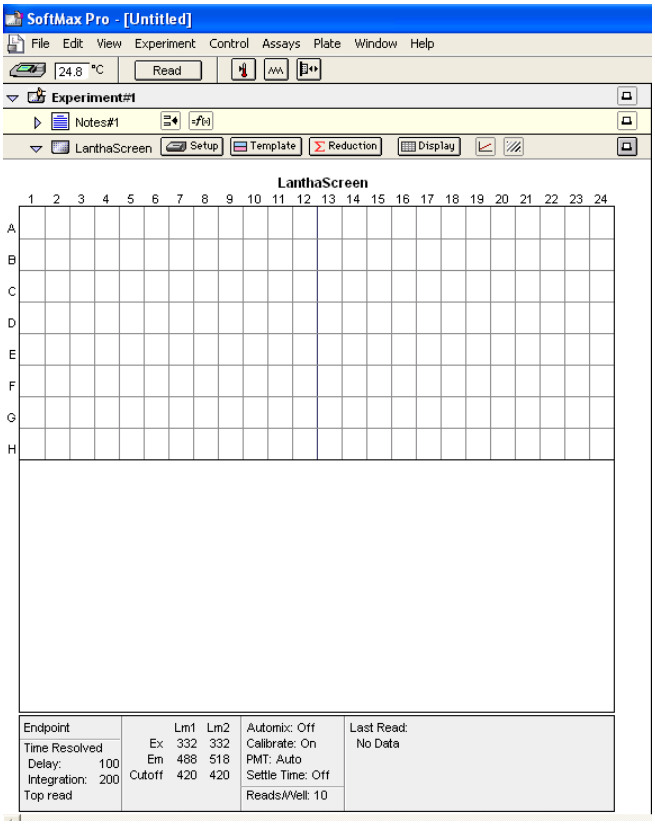


As an example, this screenshot shows information for Corning #3676 Low Volume Non-binding surface 384 well plates.

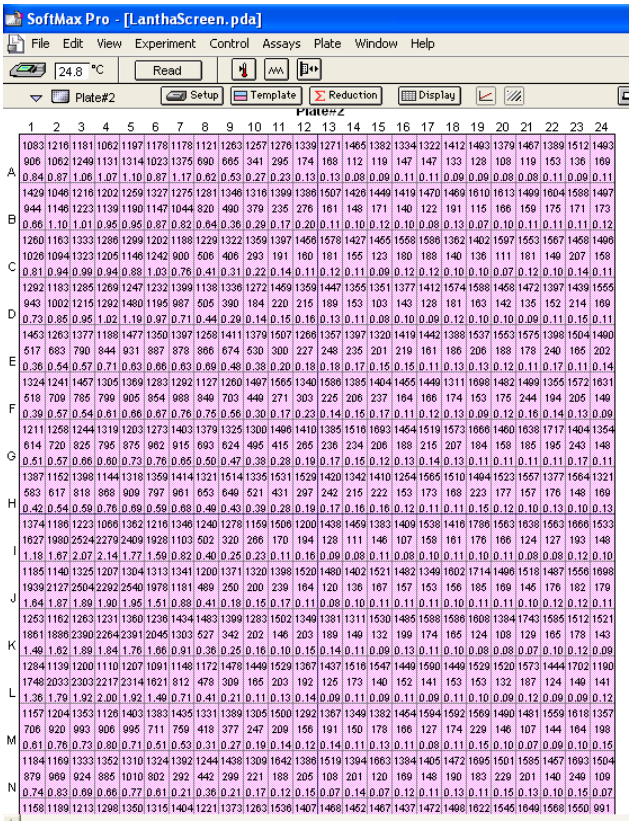
15. Select Wells to Read and move the cursor over the wells to be read. The example below shows ½ of a 384 well plate for clarity.



16. Since we do not use a settling time or use AutoRead, click OK and a blank plate format shows up:



17. Click on Read. After reading, the data is displayed for both wavelengths.

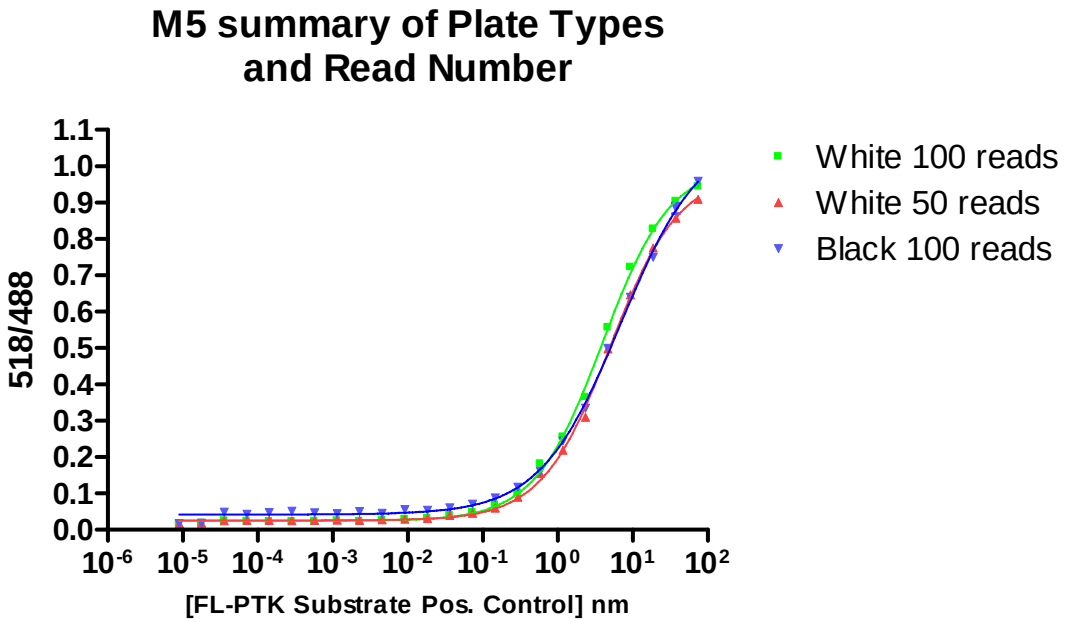


18. SoftMax Pro has data analysis capabilities that are better explained in the software tutorial. Data can be simply copied to Excel by using the Edit/Copy command and pasting into an Excel worksheet.

C. LanthaScreen® assay

Figure 1

This graph shows the results from three different reads on this reader. Fluorescein-PTK Substrate Positive Control (PV3524 is supplied with PV3552) was serially diluted in kinase buffer in either a black (Corning 3676) or a white (Corning 3673) 384 well plate. LanthaScreen® Tb-PY20 Antibody was added such that the final volume was 20µl and the Ab concentration was 2nM. The white plate was read twice, once with 100 reads/well (Sensitivity setting) and once with 50. The EC50 for all three conditions does not differ significantly in this experiment. Raw fluorescent units were up to 8 fold higher in the white plates (see Table 1), but since background RFUs were insignificant and LanthaScreen® is a ratiometric assay, data quality did not suffer in the black plates.



	White 100 reads	White 50 reads	Black 100 reads
EC50	3.986	5.008	6.898

Table 1

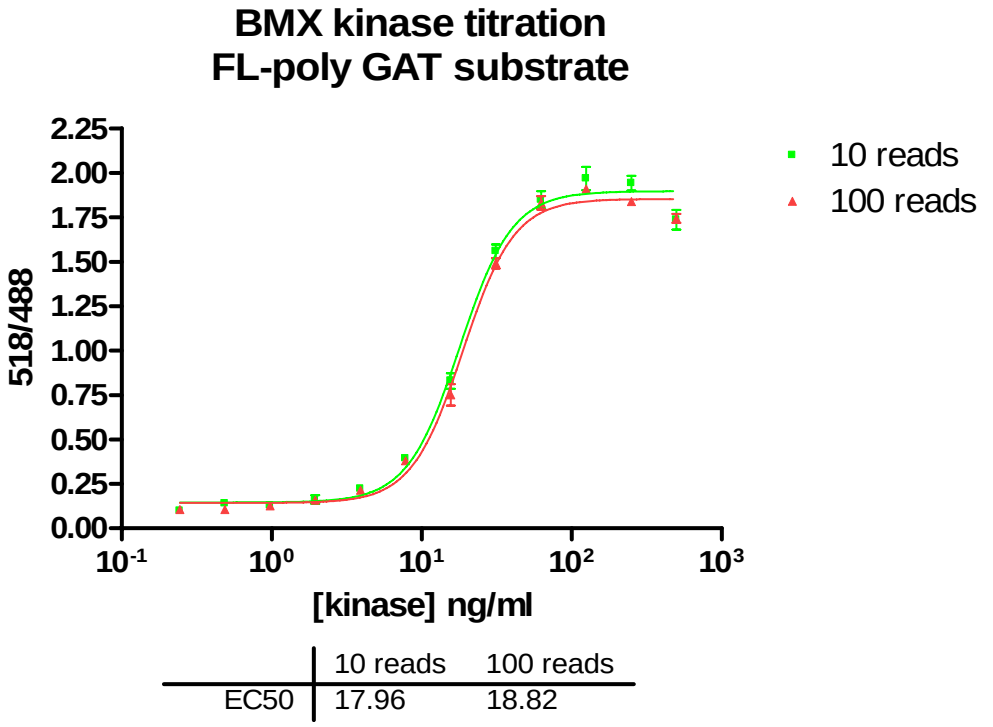
This table shows the average RFUs for the highest concentration of positive control from Figure 1 compared to the Buffer Only control at both 488 nm and 518 nm. Note that the sample/buffer ratio decreased from white to black plates but is still high enough (32 fold) to provide robust data in black plates.

	488 sample RFUs	488 buffer only RFUs	Sample/buffer	518 sample RFUs	518 buffer only RFUs	Sample/buffer

White 100 reads	6259	67	94	5910	27	215
White 50 reads	5985	79	76	5437	41	134
Black 100 reads	791	25	32	758	24	32

Figure 2

To further test sensitivity and speed of this reader, BMX (PV3371) kinase was serially diluted in the presence of Fluorescein-Poly GAT substrate (PV3611) and ATP. The kinase reaction was stopped after one hour by the addition of EDTA (10mM final) and incubated for another hour with LanthaScreen™ Tb-PY20 Antibody. The plate was read on the M5 with 100 and 10 reads/ well. EC50 calculations show no difference between the two sensitivity settings. Using 10 reads/ well reduced the read time of a full 384 well plate from 14 minutes to less than 3. Due to the robust nature of LanthaScreen® and the sensitivity of this reader, we were able to significantly reduce read time without compromising data quality.



	10 reads	100 reads
EC50	17.96	18.82