

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.

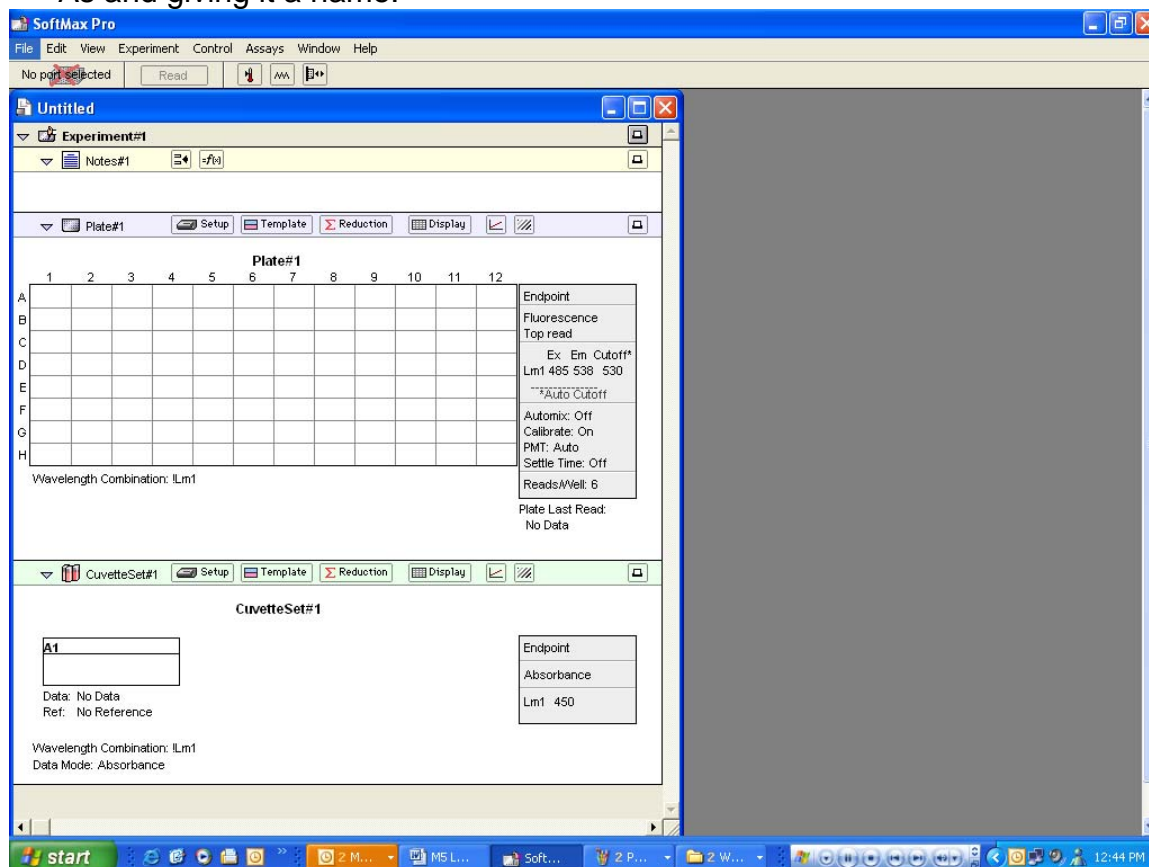
A. Recommended Optics

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

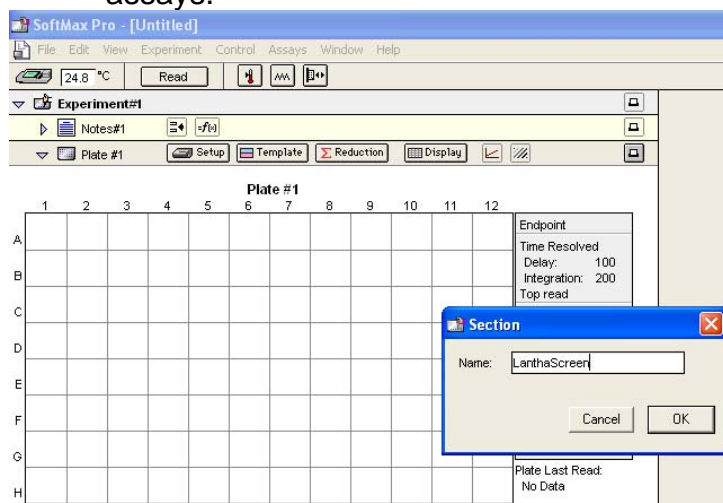
B. Instrument Setup

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.

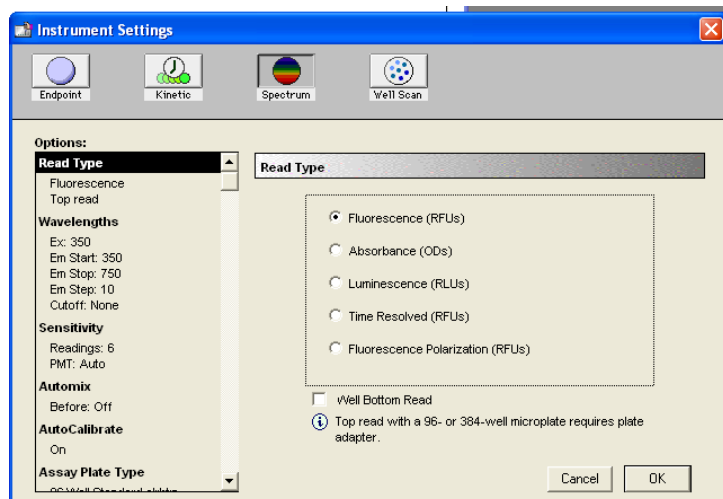


2. You can double click on Plate #1 and rename this section as shown in the example below. This is not necessary but could be helpful in order to keep track of different assays.

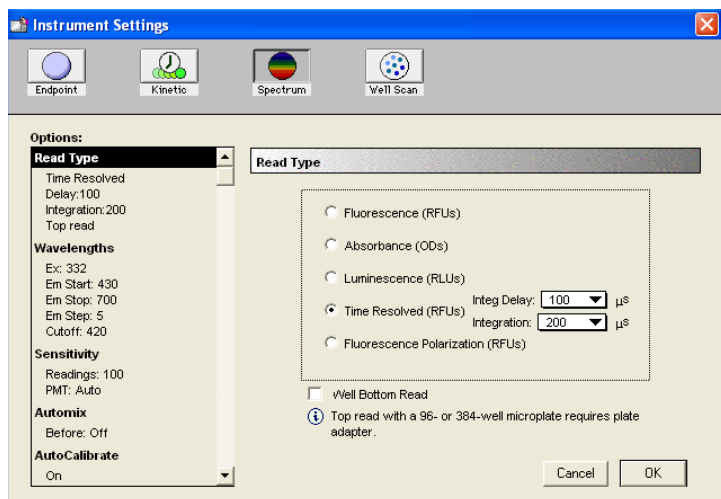


3. We find it convenient to run a wavelength spectrum from a well that contains only the Tb labeled antibody. This gives you visual proof that the wavelengths you are using are the optimal. Skip to step # 11 if you do not want to do a Spectrum analysis.

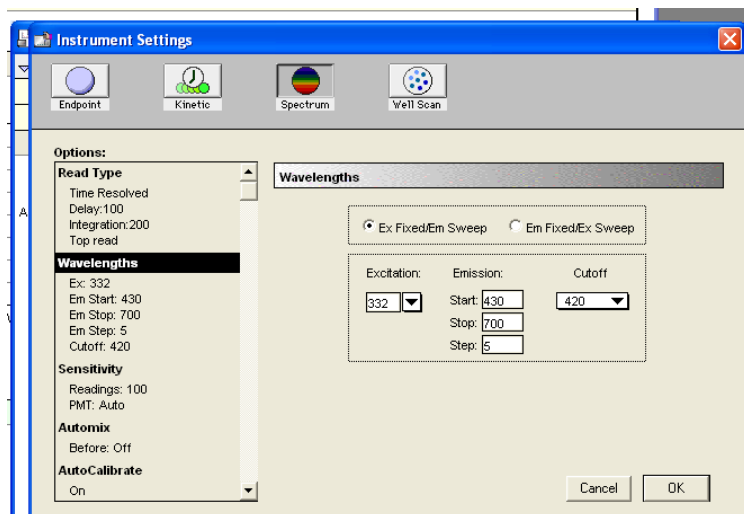
Click on the Set Up icon right next to LanthaScreen® and the following screen appears:



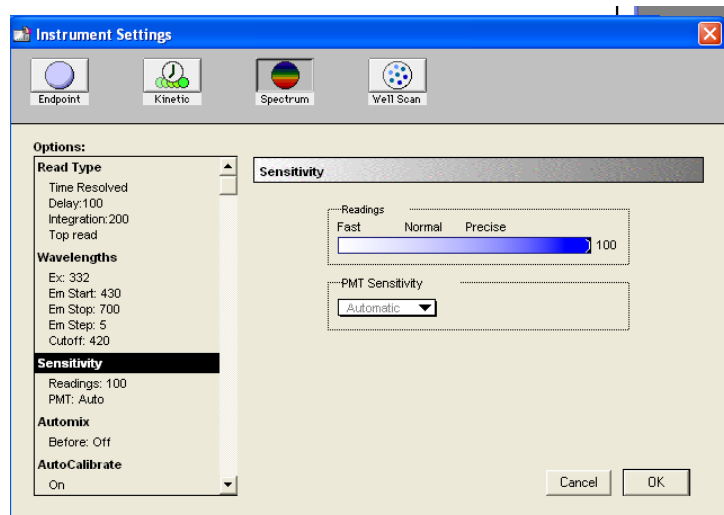
4. Check Time Resolved and enter the values shown below (Integ Delay 100 μ s, Integration = 200 μ s)



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).



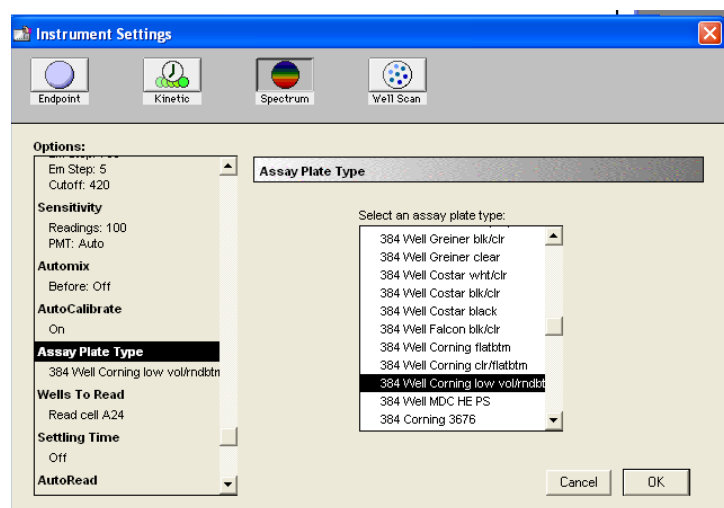
6. Click on Sensitivity and move the slider all the way to the right for optimal sensitivity (=100). Note that PMT Sensitivity = Automatic is the only choice for Spectrum.



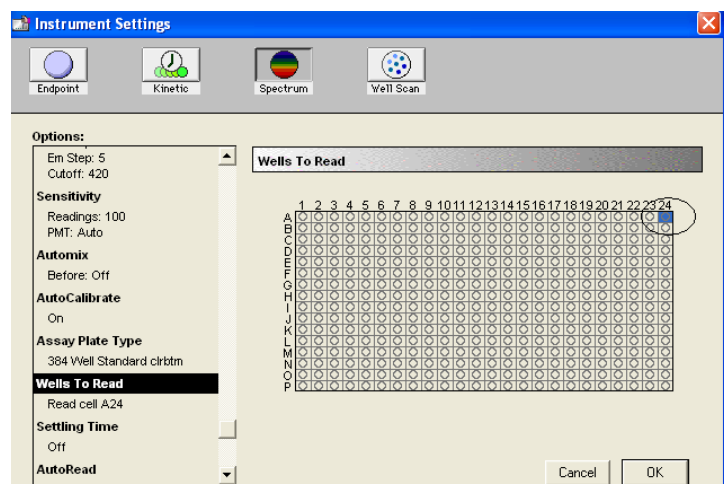
7. Using the slide bar, bypass Automix, make sure AutoCalibrate is chosen



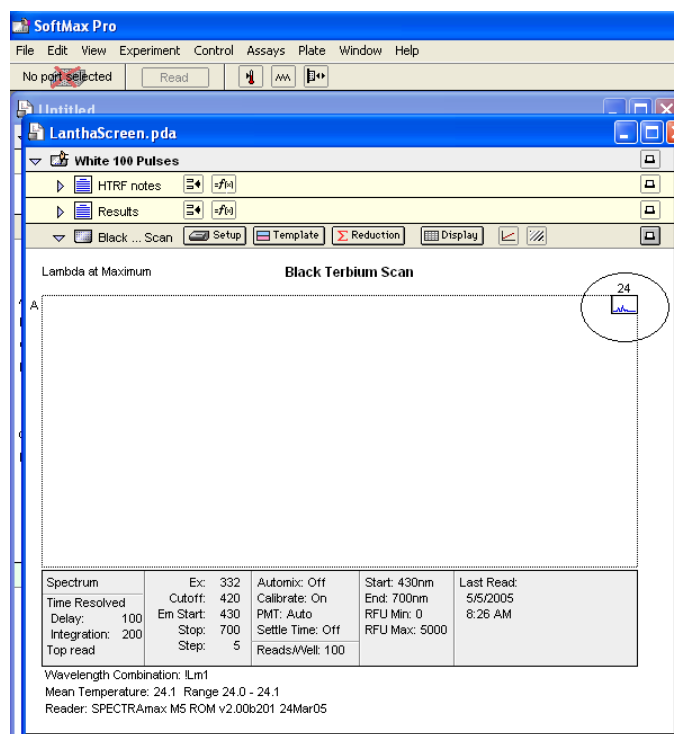
and double click on Assay Plate type. Choose the appropriate plate from the drop down menu.



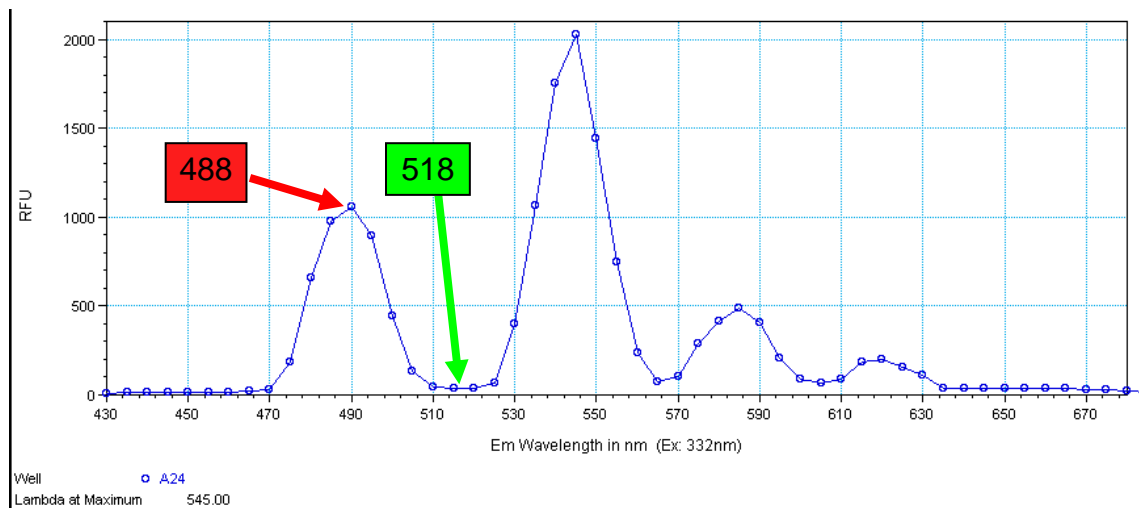
8. Click on Wells To Read and choose one well containing only the Tb labeled Ab. In the example below, we have chosen A24.



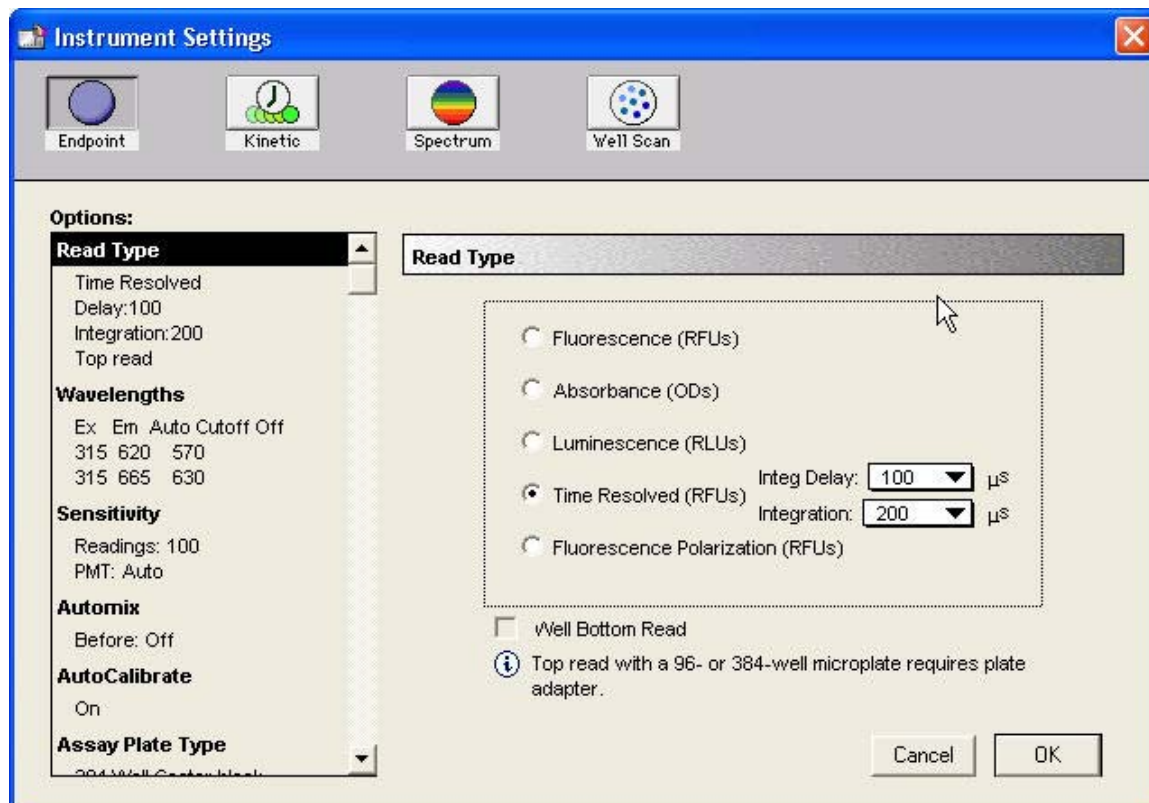
9. Click on OK and then Read. After the read, the following screen will appear:



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 nm for the acceptor.

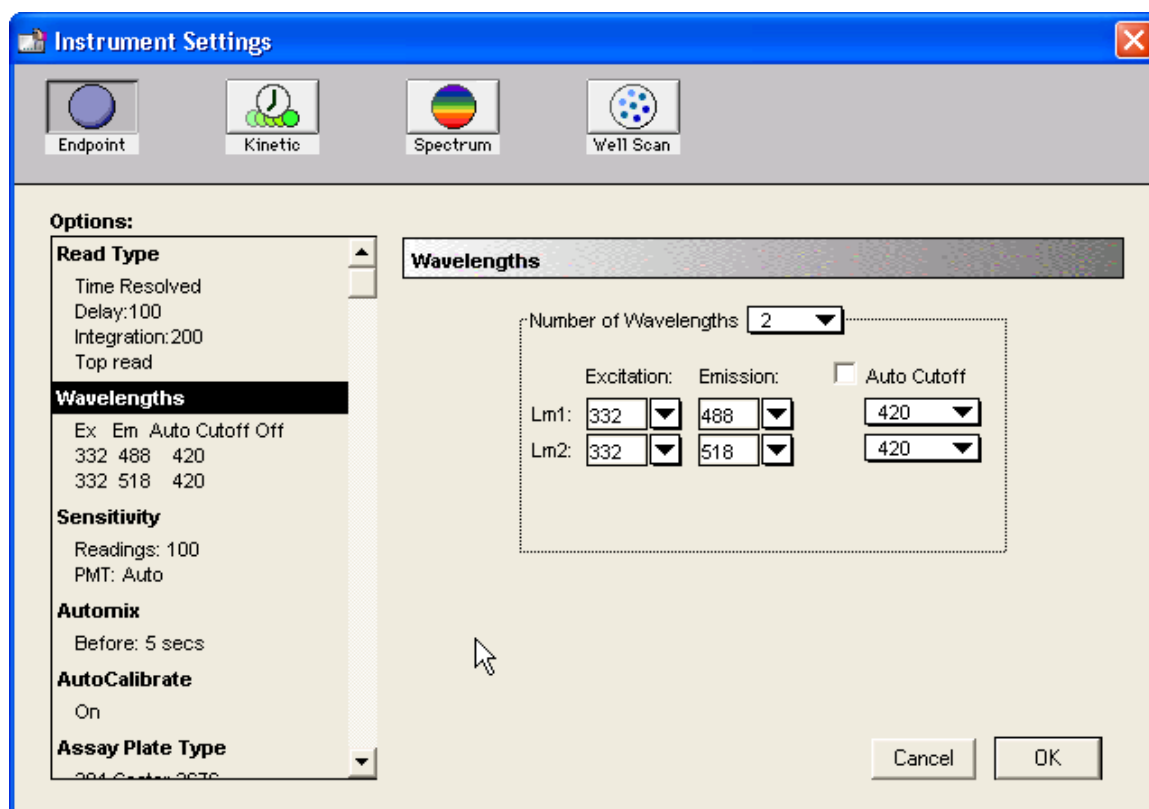


11. Click on the Set Up icon right next to LanthaScreen® and the following screen appears:
 - a. Click on Endpoint, followed by Read Type (under Options).
 - b. Click on Time Resolved and enter 100 for Integ Delay and 200 for Integration.

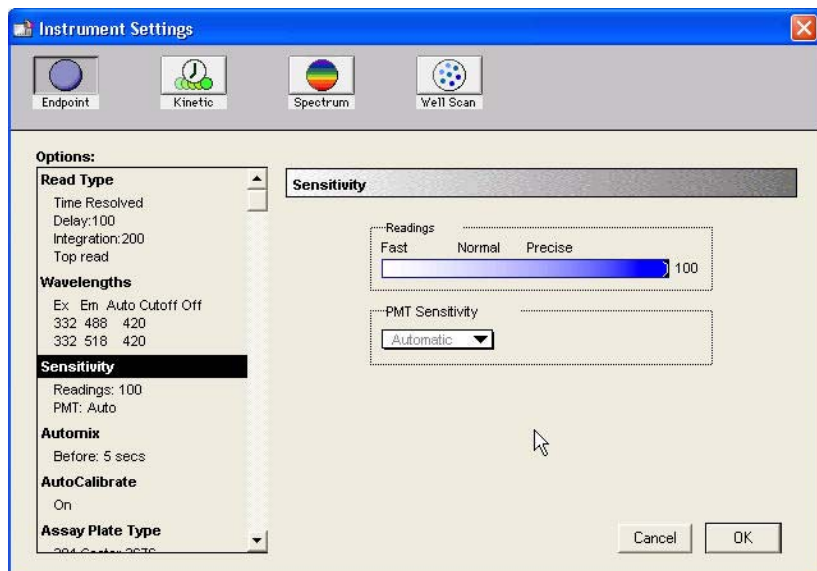


12. Click on Wavelengths and enter the following information: Number of Wavelengths = 2; Lm1 Excitation = 332, Emission = 488; Lm2 Excitation = 332, Emission = 518; Cutoff for both Lm1 and Lm2 = 420 (unclick Auto Cutoff to enable the cutoff choices). If the wavelength or cutoff is not one of the default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

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



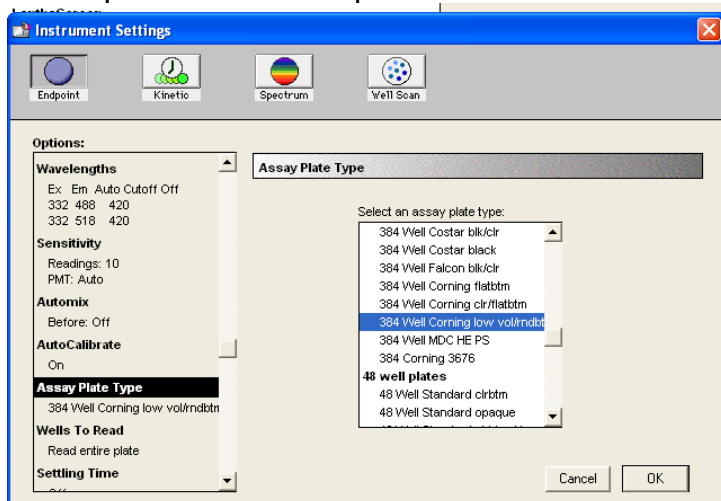
13. Click on Sensitivity and move the slider until it reads 100, all the way to the right. This is the most sensitive setting (100 reads/well) and therefore takes the most time (~15 minutes/384 well plate). We have tried the same plate at 100 reads and at 10 reads with very little difference in data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



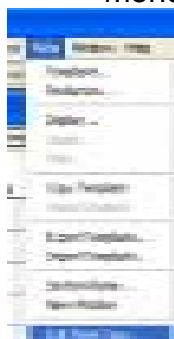
- 14a. Using the slide bar, bypass Automix, make sure AutoCalibrate is chosen



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



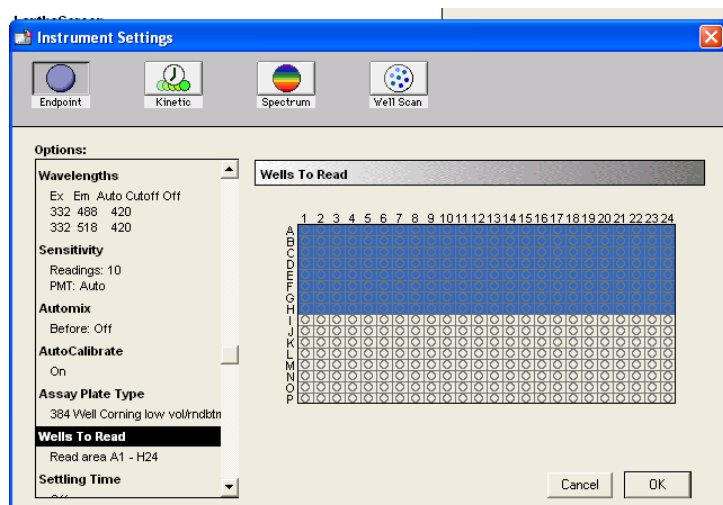
- 14b. If you need to enter plate information, select Edit Plate Type from the Plate drop down menu.



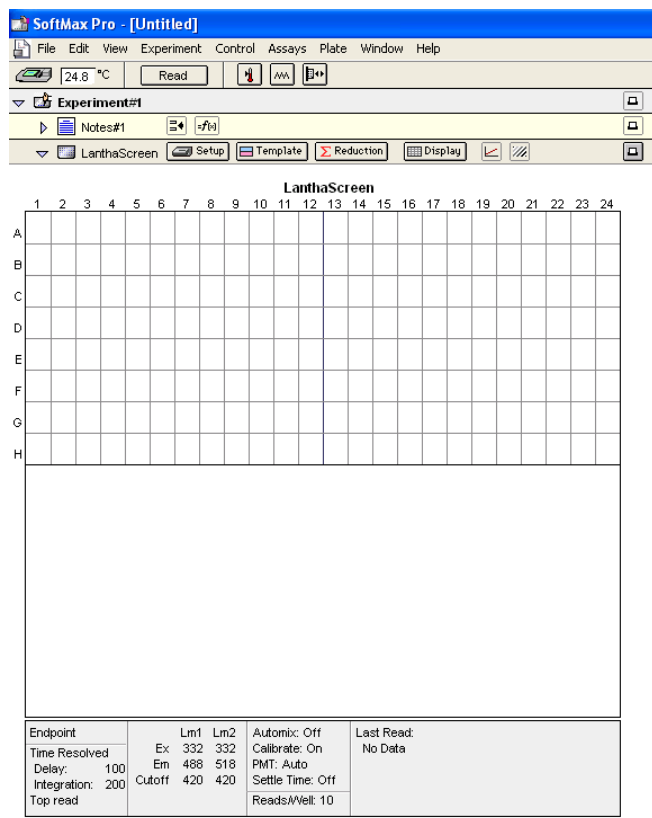
As an example, this screenshot shows information for Corning #3676 Low Volume Non-binding surface 384 well plates.



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.

SoftMax Pro [LanthaScreen.pda]

File Edit View Experiment Control Assays Plate Window Help

24.8 °C Read

Plate#2

Template Reduction Display

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1083	1216	1181	1062	1197	1178	1178	1121	1263	1257	1276	1339	1271	1465	1382	1334	1322	1412	1463	1379	1467	1389	1512	1493
B	906	1062	1249	1131	1314	1023	1375	690	665	341	295	174	188	112	119	147	147	133	128	108	119	153	136	169
C	0.84	0.87	1.06	1.10	0.87	1.17	0.62	0.53	0.27	0.23	0.13	0.13	0.08	0.09	0.11	0.11	0.09	0.09	0.08	0.11	0.09	0.11	0.11	
D	1429	1048	1216	1202	1259	1327	1275	1281	1346	1316	1399	1388	1507	1426	1449	1419	1470	1469	1610	1613	1469	1604	1588	1497
E	944	1148	1223	1139	1147	1044	820	490	379	235	276	181	148	171	149	122	191	116	166	159	175	171	173	
F	0.86	1.10	1.01	0.95	0.95	0.87	0.82	0.64	0.36	0.29	0.17	0.20	0.11	0.10	0.12	0.10	0.08	0.13	0.07	0.10	0.11	0.11	0.12	
G	1260	1163	1333	1295	1299	1202	1188	1229	1322	1359	1397	1468	1578	1427	1455	1558	1586	1362	1402	1597	1553	1567	1458	1496
H	1028	1094	1316	1148	1242	900	506	406	293	191	180	181	155	123	180	188	140	136	111	181	149	207	158	
I	0.81	0.94	0.99	0.94	0.88	1.03	0.76	0.41	0.31	0.22	0.14	0.11	0.12	0.11	0.09	0.12	0.12	0.10	0.10	0.07	0.12	0.10	0.14	0.11
J	1292	1183	1285	1269	1247	1232	1399	1138	1336	1272	1459	1359	1447	1355	1351	1377	1412	1574	1588	1458	1472	1397	1439	1555
K	943	1002	1215	1292	1480	1195	987	505	390	184	220	215	189	153	103	143	128	181	163	142	135	152	214	169
L	0.73	0.85	0.95	1.02	1.19	0.97	0.71	0.44	0.29	0.14	0.15	0.16	0.13	0.11	0.09	0.10	0.09	0.12	0.10	0.10	0.09	0.11	0.15	0.11
M	1463	1263	1377	1188	1477	1350	1367	1258	1411	1379	1507	1268	1357	1397	1320	1419	1442	1388	1537	1553	1575	1398	1504	1460
N	517	683	790	844	931	887	878	866	674	530	300	227	248	235	201	219	161	186	206	188	178	240	165	202
O	0.36	0.54	0.57	0.71	0.63	0.66	0.63	0.69	0.48	0.38	0.20	0.18	0.18	0.17	0.15	0.15	0.11	0.13	0.13	0.12	0.11	0.17	0.11	0.14
P	1324	1241	1467	1305	1369	1263	1262	1127	1260	1467	1565	1340	1588	1385	1404	1455	1449	1311	1698	1462	1469	1355	1572	1631
Q	518	708	785	796	905	854	968	848	703	449	271	303	225	206	237	164	166	174	153	175	244	194	205	148
R	0.39	0.57	0.54	0.61	0.66	0.67	0.76	0.75	0.56	0.30	0.17	0.23	0.14	0.15	0.17	0.11	0.12	0.13	0.09	0.12	0.16	0.14	0.13	0.09
S	1211	1268	1244	1319	1203	1273	1403	1379	1325	1300	1496	1410	1385	1516	1693	1454	1519	1573	1666	1460	1638	1717	1464	1354
T	614	720	825	795	875	962	915	693	624	495	415	265	236	234	206	168	215	207	184	158	185	195	243	148
U	0.51	0.57	0.66	0.89	0.73	0.76	0.65	0.50	0.47	0.38	0.20	0.19	0.17	0.15	0.12	0.13	0.14	0.15	0.11	0.11	0.11	0.17	0.11	0.17
V	1307	1152	1398	1144	1316	1359	1414	1221	1514	1335	1531	1529	1420	1342	1410	1254	1565	1510	1494	1523	1557	1377	1564	1321
W	563	617	818	986	909	797	981	653	649	521	431	297	242	215	222	153	173	188	223	177	157	176	148	169
X	0.42	0.54	0.59	0.76	0.69	0.59	0.68	0.49	0.43	0.39	0.28	0.19	0.17	0.16	0.16	0.12	0.11	0.11	0.15	0.12	0.10	0.13	0.10	0.13
Y	1374	1186	1223	1068	1362	1216	1346	1240	1278	1159	1508	1200	1436	1459	1363	1469	1538	1416	1786	1563	1638	1663	1686	1533
Z	1827	1980	2524	2279	2409	1928	1103	502	320	268	170	194	128	111	146	107	158	161	178	166	124	127	193	148
AA	1.18	1.87	2.07	2.14	1.77	1.59	0.82	0.40	0.25	0.23	0.11	0.18	0.09	0.09	0.11	0.09	0.10	0.11	0.10	0.11	0.08	0.08	0.12	0.10
AB	1195	1140	1225	1207	1304	1313	1341	1200	1371	1320	1398	1520	1480	1402	1521	1462	1349	1602	1714	1498	1518	1487	1556	1698
AC	1939	2127	2504	2292	2540	1978	1101	489	250	200	239	184	120	136	167	157	153	156	185	169	146	176	182	179
AD	1.64	1.87	1.89	1.90	1.95	1.51	0.89	0.41	0.18	0.15	0.17	0.11	0.08	0.10	0.11	0.11	0.10	0.11	0.10	0.11	0.10	0.12	0.12	0.11
AE	1253	1162	1263	1231	1260	1236	1404	1403	1399	1283	1502	1349	1381	1311	1530	1485	1588	1588	1608	1394	1743	1695	1512	1521
AF	1881	1886	2390	2264	2391	2045	1303	527	342	202	146	203	189	149	132	199	174	165	124	108	129	165	178	143
AG	1.49	1.62	1.89	1.84	1.76	1.66	0.91	0.38	0.25	0.19	0.10	0.15	0.14	0.11	0.09	0.13	0.11	0.10	0.08	0.08	0.07	0.10	0.12	0.09
AH	1284	1139	1200	1110	1207	1091	1148	1172	1478	1449	1529	1367	1437	1516	1547	1449	1590	1449	1529	1520	1573	1444	1702	1190
AI	1748	2033	2303	2217	2314	1621	812	478	309	165	203	192	125	173	140	152	141	153	153	132	187	124	149	141
AJ	1.36	1.79	1.92	2.00	1.92	1.49	0.71	0.41	0.21	0.11	0.13	0.14	0.09	0.11	0.09	0.11	0.10	0.09	0.12	0.09	0.09	0.12	0.09	0.12
AK	1157	1204	1353	1126	1403	1383	1435	1331	1389	1305	1500	1292	1367	1349	1382	1454	1594	1592	1569	1490	1481	1559	1618	1357
AL	706	820	993	906	995	711	759	418	377	247	209	156	191	150	178	196	127	174	229	146	107	144	164	198
AM	0.61	0.76	0.73	0.80	0.71	0.51	0.53	0.31	0.27	0.19	0.14	0.12	0.14	0.11	0.13	0.11	0.09	0.11	0.15	0.10	0.07	0.09	0.10	0.15
AN	1184	1169	1333	1352	1310	1324	1392	1244	1438	1309	1642	1398	1519	1394	1663	1384	1405	1472	1695	1501	1585	1457	1693	1504
AO	879	999	924	885	1010	802	292	442	299	221	188	205	108	201	120	169	148	190	183	229	201	140	240	109
AP	0.74	0.83	0.69	0.66	0.77	0.61	0.21	0.38	0.21	0.17	0.12	0.15	0.07	0.14	0.07	0.12	0.11	0.13	0.11	0.15	0.13	0.10	0.15	0.07
AP	1158	1189	1213	1298	1350	1315	1404	1221	1373	1263	1536	1407	1468	1452	1467	1437	1472	1498	1622	1545	1649	1568	1550	991

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.

**M5 summary of Plate Types
and Read Number**

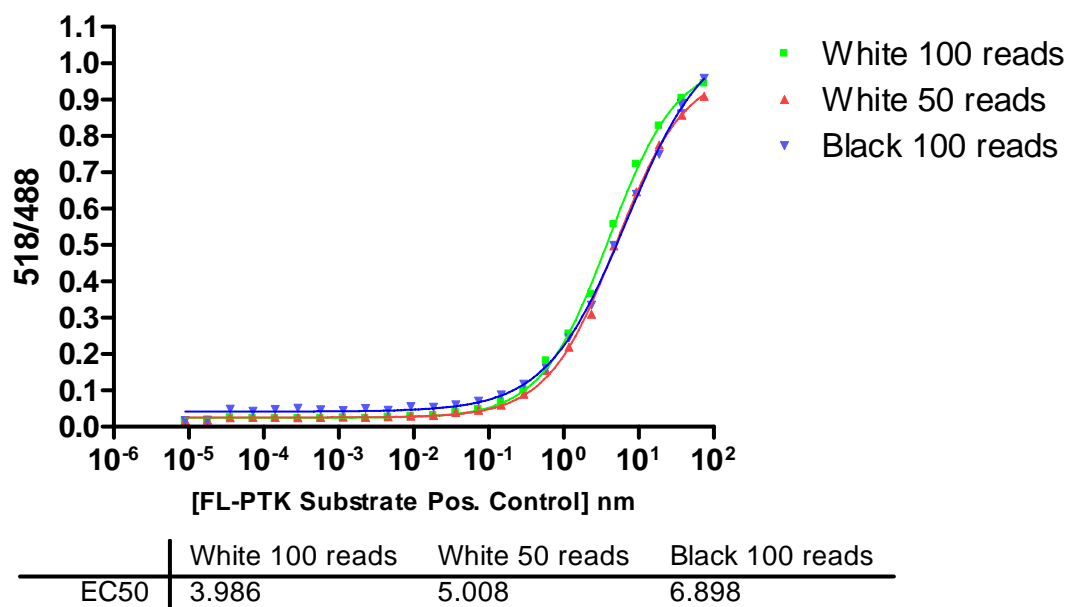


Table 1

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.

BMX kinase titration FL-poly GAT substrate

