Predictor™ Assay Setup Guide on the PerkinElmer EnVision® Multilabel Reader

The PerkinElmer EnVision® Multilabel Reader was tested for compatibility with Life Technologies Predictor™ hERG FP Assay using controls provided within the assay kit and the known hERG channel blockers astemizole and terfenadine. The following document is intended to demonstrate setup of this instrument and provide representative data.

For more detailed information and technical support of Life Technologies assays including specific conditions for assay windows between 2-3 fold, please call 1-800-955-6288 and enter extension 40266 or email drugdiscoverytech@lifetech.com.

For more detailed information and technical support of PerkinElmer instruments or software, please call 1-800-762-4000 or by e-mail at productinfo@perkinelmer.com .

Setup Guide on the PerkinElmer EnVision® Multilabel Reader

A. Recommended Optics

| PerkinElmer Part Number	Wavelength (nm)	Diameter (mm)
Excitation (#2100-5050)	531/25	15 (BODIPY TMR FP filter)
Emission 1 (S-pol #2100-5800)	579/25	15
Emission 2 (P-pol #2100-5810)	579/25	15
Dichroic Mirror (#2100-4080)	D555fp/D595	(BODIPY TMR FP dual)*

Note: Original validation was performed using Emission filters #2100-5160 and 2100-5170, S- and P-pol 595/60 filters. These filters were less optimal than the filters listed above, which give a larger assay window and improved Z' values.

*We have also tested a D555fp/580 dual dichroic mirror (custom) from PE, which also performs with Predictor assays, as well as a D55FP.
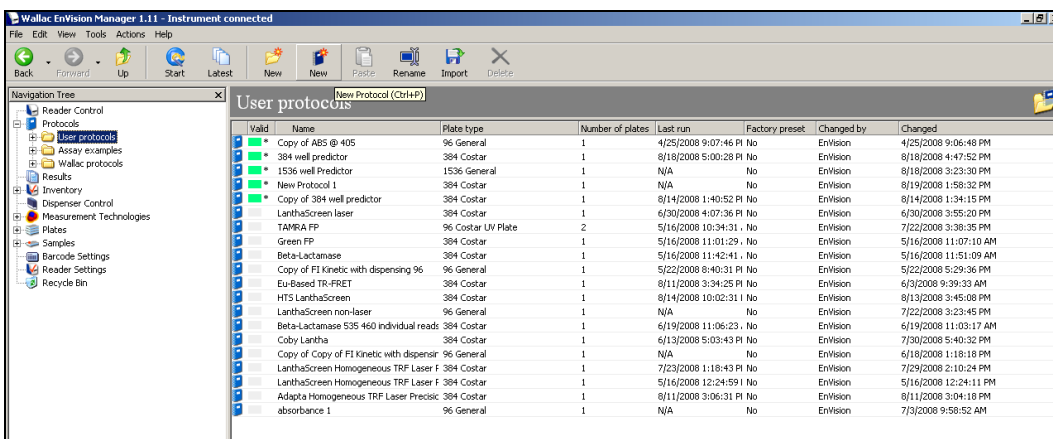
B. Instrument Setup

1. Make certain plate reader is turned on, and open up Wallac EnVision® Manager software on computer.

2. When Wallac EnVision® Manager opens, if you do not have a pre-existing protocol for Predictor™, select "Protocol" in the "Navigation Tree" menu near the upper left corner of the screen.
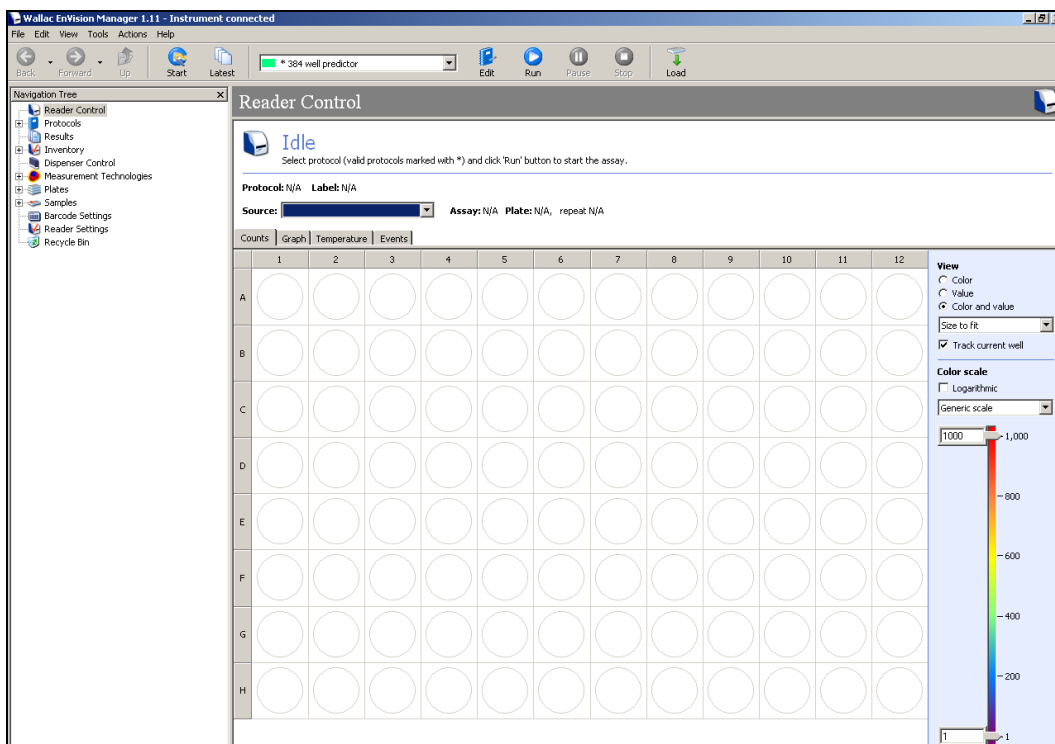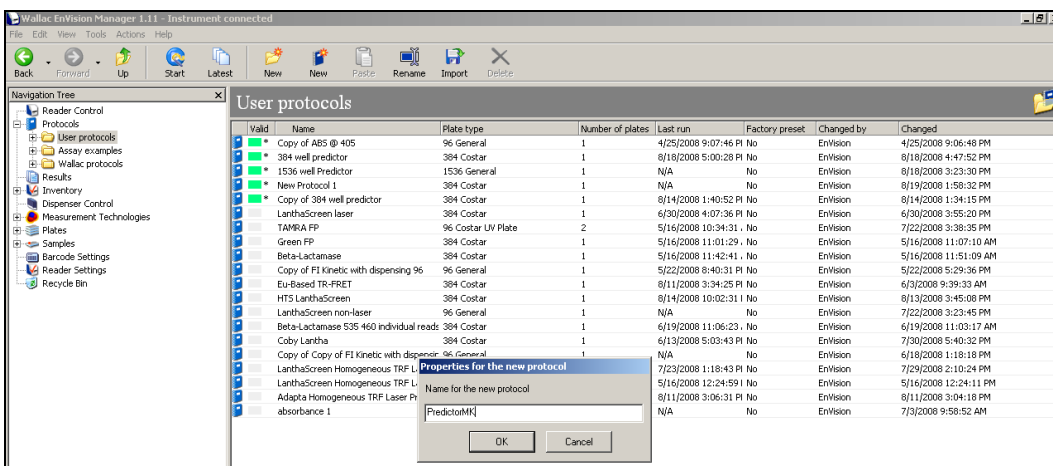
3. At this point, a protocol list will open (below). Assuming there is no Predictor™ or similar FP-Red protocol set up, click on the blue "New Protocol" button on the toolbar at the top of the screen.

4. Follow the prompt and enter a name for the protocol, then select "OK".

Predictor™ Compatible Microplate
Reader Documentation

Version No.:
13 April 12

Page 5 of 19

Setup Guide on the PerkinElmer EnVision® Multilabel Reader

5.   Your new protocol will open automatically. **Note**: Several things will need to be changed and a preliminary warning may appear as below under "Measurement" (in this case because filters for the new assay need to be defined).
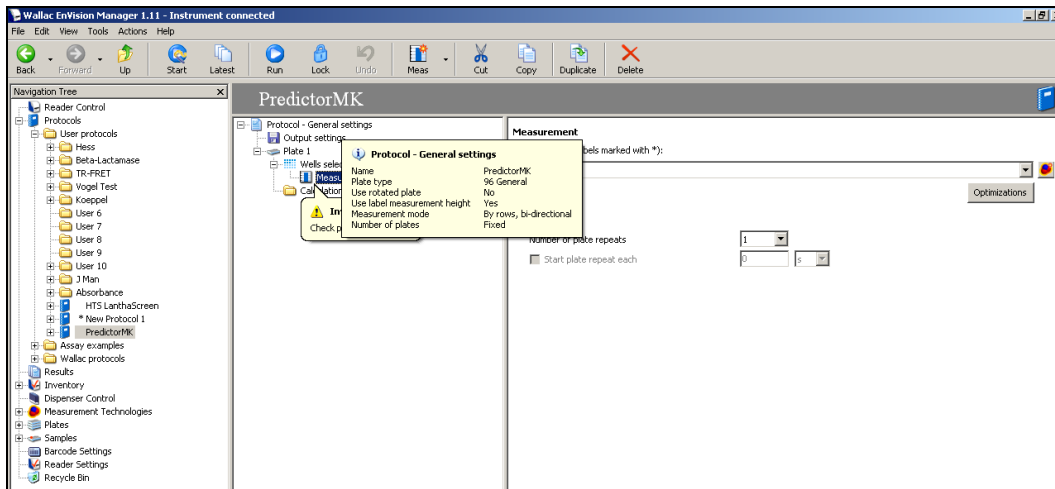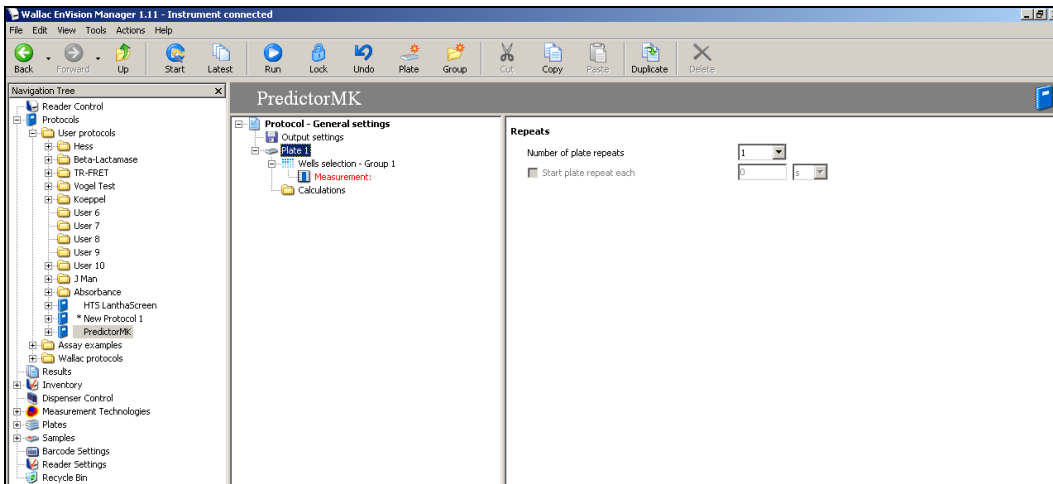

6.   From the new Protocol menu near the center of the screen select "Protocol General settings". Choose your plate type. **Note**: For this protocol we selected "384 Costar".
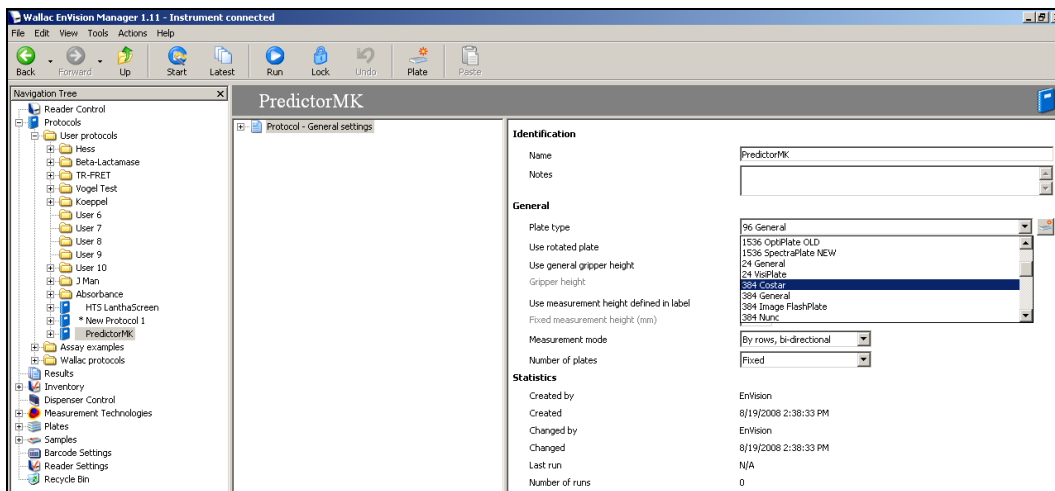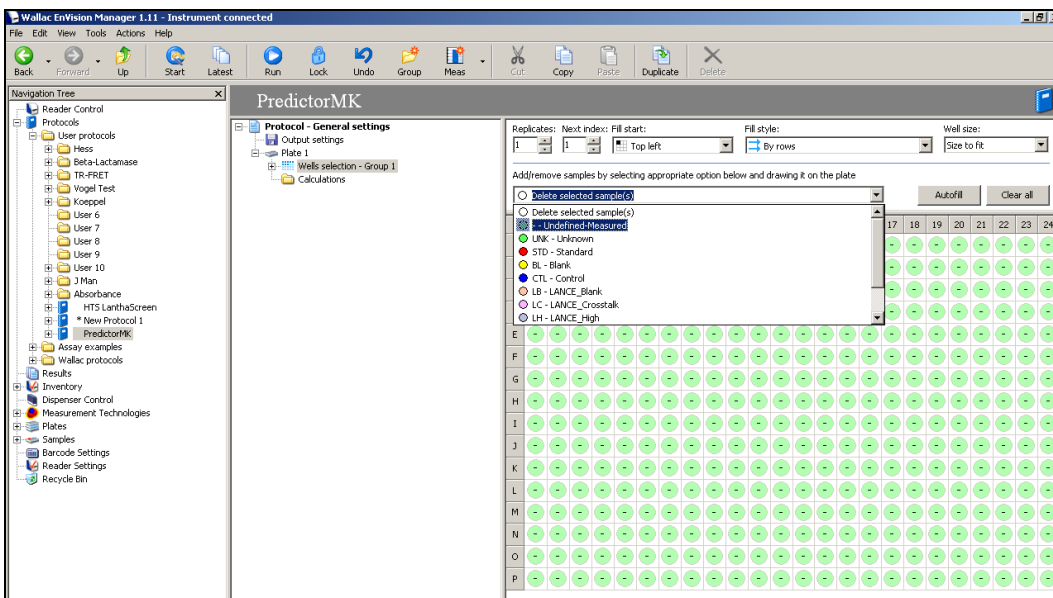
Setup Guide on the PerkinElmer EnVision® Multilabel Reader

7. Select "Plate 1", set "Number of Repeats" to 1.

8. Now select "Well Selection", using the drop-down toolbar to define your wells as needed.

9. **Note**: In this example and the setup for the experiment below, Column 16 of the plate is all blanks. The EnVision® software performs much better in our hands when blanks are selected for auto-subtraction as in the figure below before the assay is run, and for all readers blank subtraction typically results in a 10-20% increase in assay window. A blank consisting of only the Assay Buffer is used as the blank for the Free Tracer Control. An Assay Blank, consisting of Membrane and Assay Buffer is used as the blank for test compounds.
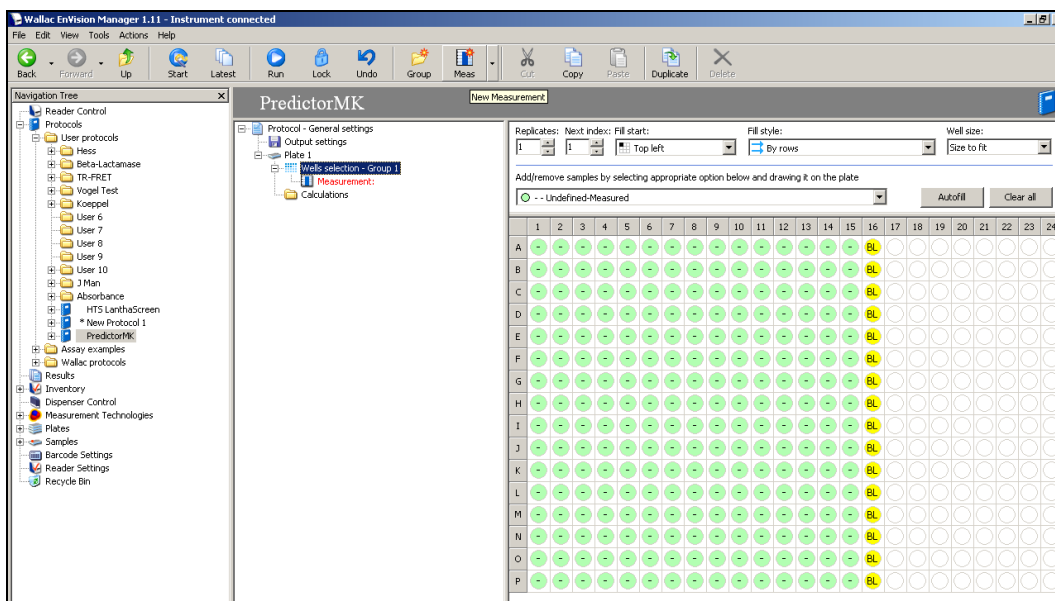
10. Select "Measurement" in the Protocol window, and select the correct filter set. **Note**: PerkinElmer offers a "*FP-FP Bodipy dual" set which we used here.
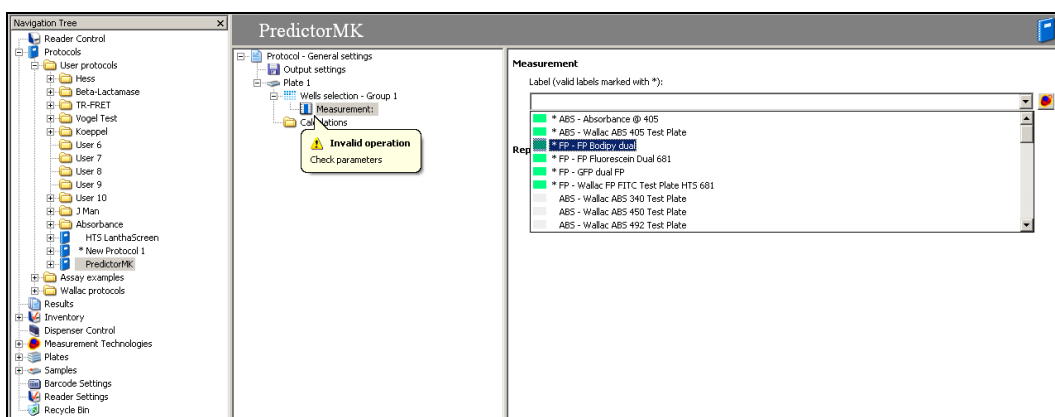
Setup Guide on the PerkinElmer EnVision® Multilabel Reader

11. Left-click on "Calculations" in the Protocol toolbar (partially obscured here) and select the relevant calculations you would like to perform. We recommend selecting "#6-mP value for FP measurement", "#8-mP value (Blank corrected, blanks on each plate), and "#10-Total fluorescence intensity".

Setup Guide on the PerkinElmer EnVision® Multilabel Reader

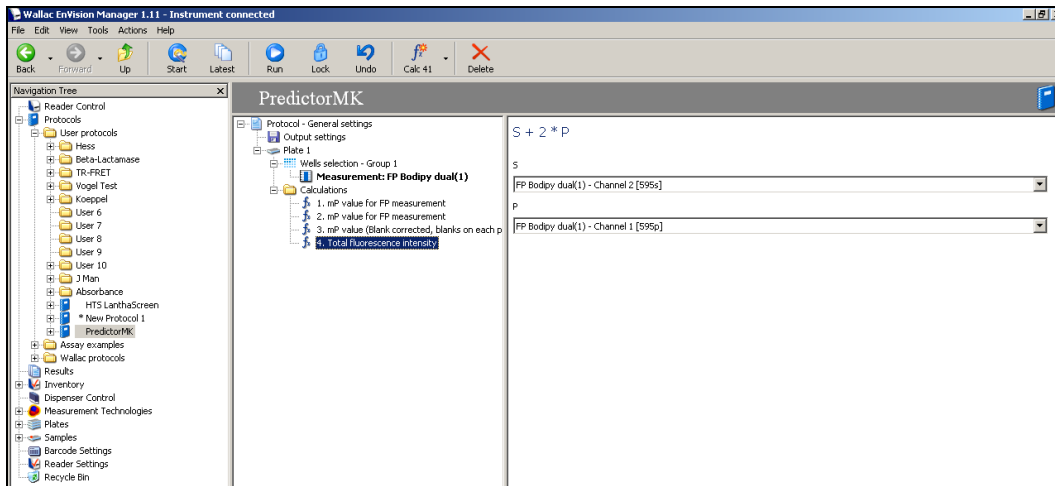12. Once all calculations are selected, click on "Start" on the toolbar at the top of the screen (not "Run").

13. This will open the Assay Start Wizard. Click "Next".
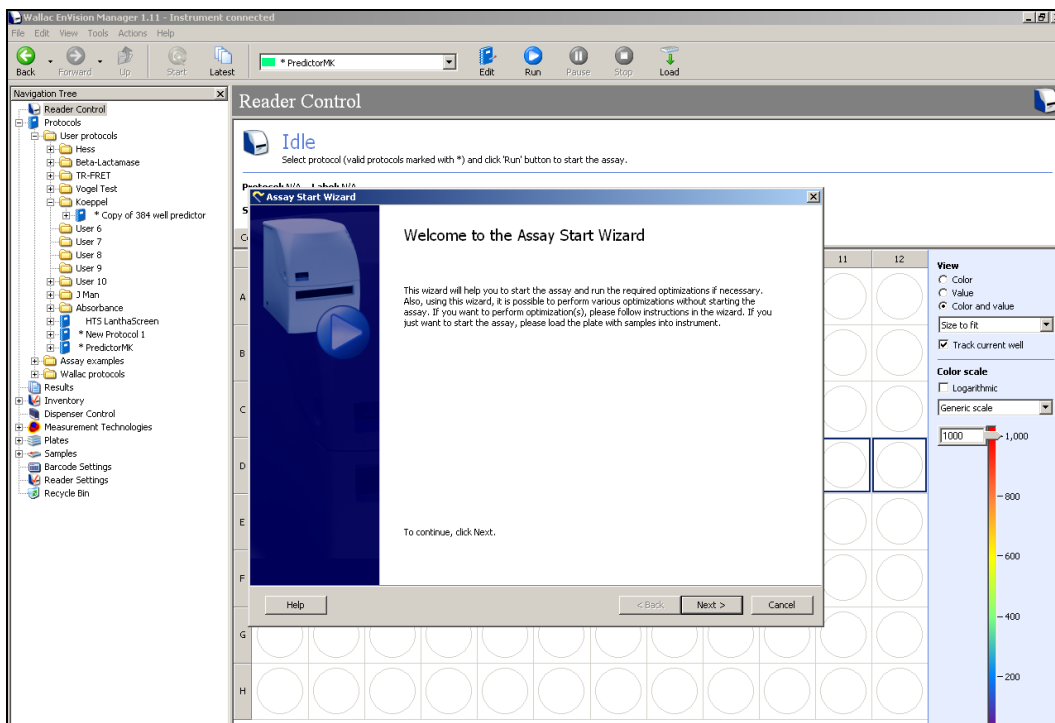
Setup Guide on the PerkinElmer EnVision® Multilabel Reader

14. Select your protocol from the list as the protocol you wish to optimize, and then click "Next".
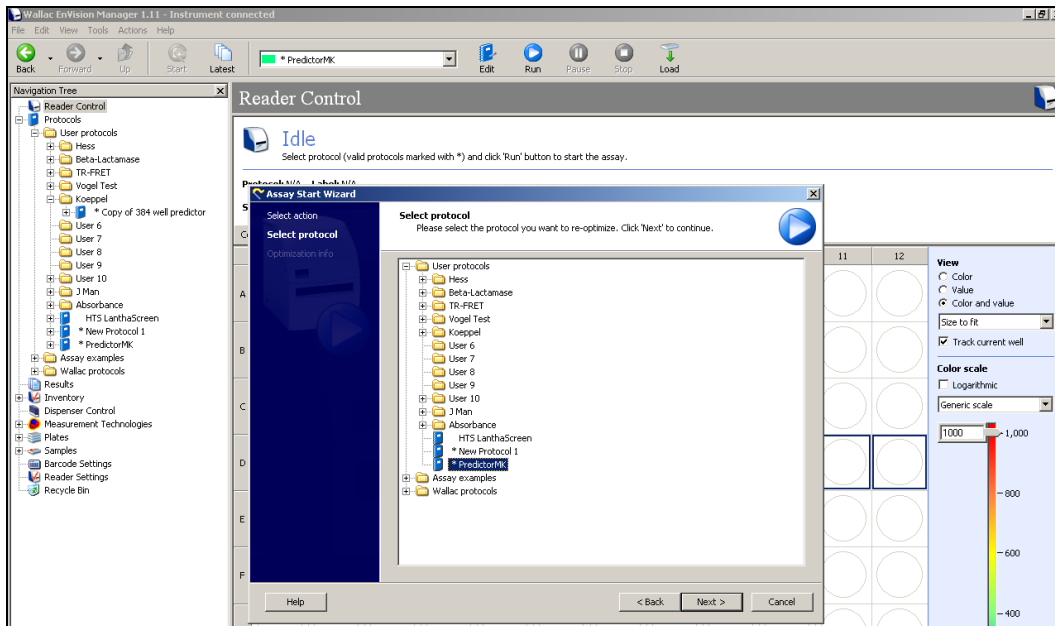
15. A new screen will open. Select "Measurement Gain" and "Detector Gain" by clicking in each check box, and enter "50" in the "Target mP Value" box, then select "Next".
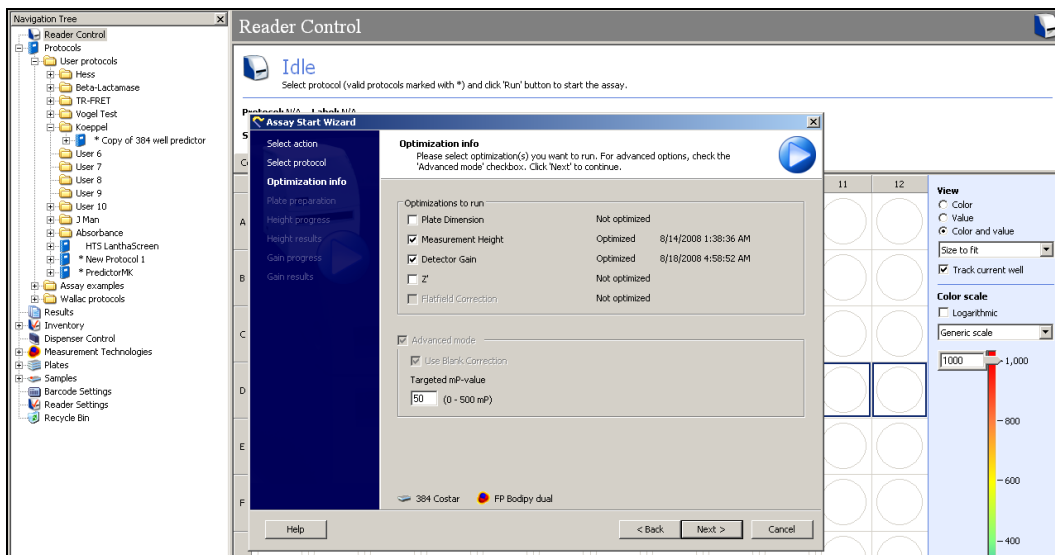
Have a question?	NA: 800-955-6288 or INTL: 760-603-7200 ext. 40266
Contact our Technical Support Team	Email: drugdiscoverytech@lifetech.com

Setup Guide on the PerkinElmer EnVision® Multilabel Reader

16. A new plate layout screen will appear. Enter "100" in the "Number of flashes" box at the right, and select appropriate wells for determining "Height", "Gain," and "Blank". In this case "Blank" was chosen from Column 16 (use the Buffer Blank), and Gain was set from Column 15 (Free Tracer column). When finished, select "Next".

17. The EnVision® will now begin to run through the Assay Wizard, starting with the Height optimization. When the prompt appears indicating the Height optimization is finished, select "Next".
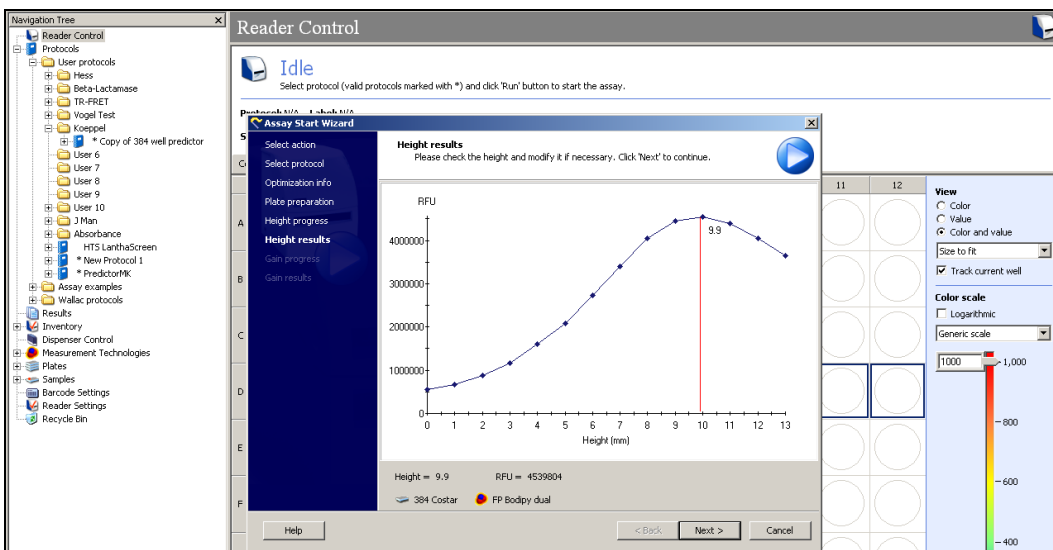
Setup Guide on the PerkinElmer EnVision® Multilabel Reader

18. The instrument will now calculate the Gain. A prompt will appear showing the results. Select "Next".
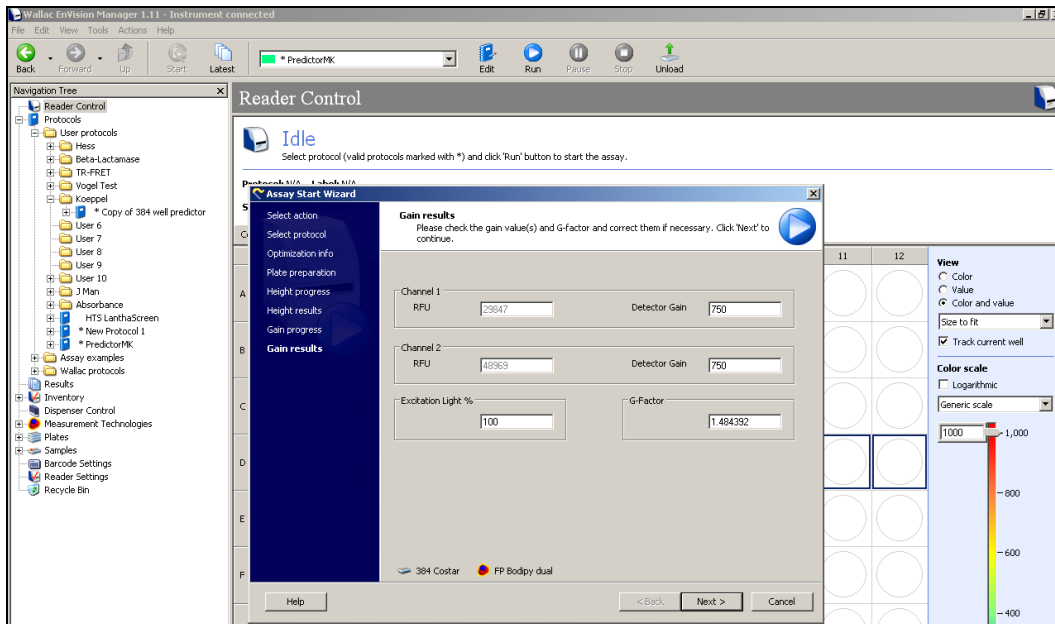
19. The Wizard is finally finished, the following prompt will appear. Click on "Finish" and instrument will begin to read your assay plate.
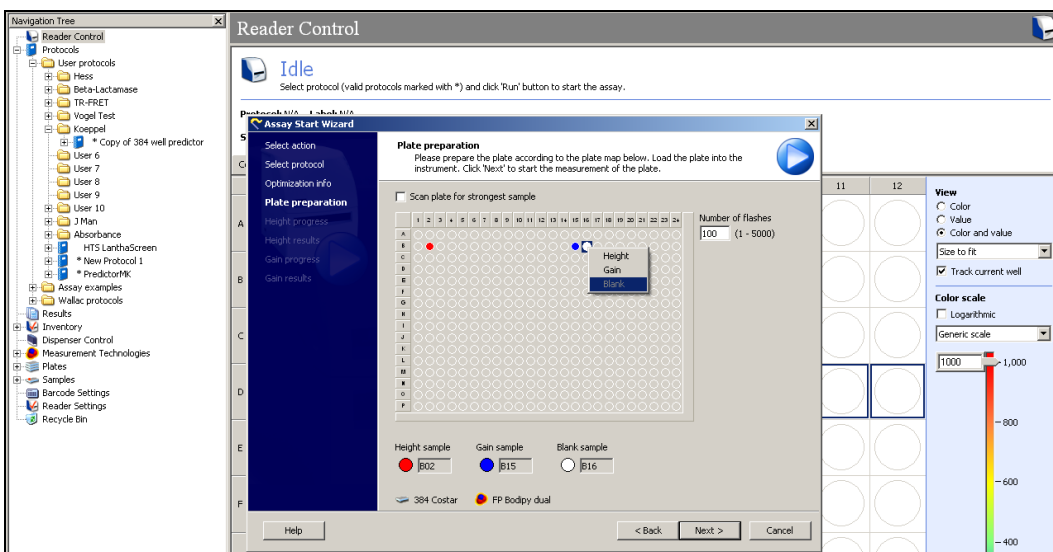

Have a question?	NA: 800-955-6288 or INTL: 760-603-7200 ext. 40266
Contact our Technical Support Team	Email: drugdiscoverytech@lifetech.com

	Predictor™ Compatible Microplate Reader Documentation	Version No.: 13 April 12	Page 13 of 19

Setup Guide on the PerkinElmer EnVision® Multilabel Reader

20. As the EnVision® reads the assay plate, it will populate values along a plate layout schematic on-screen. **Note:** The screenshot below shows "mP value (not blank-corrected)"—you can also select "mP value (Blank-Corrected)" here.
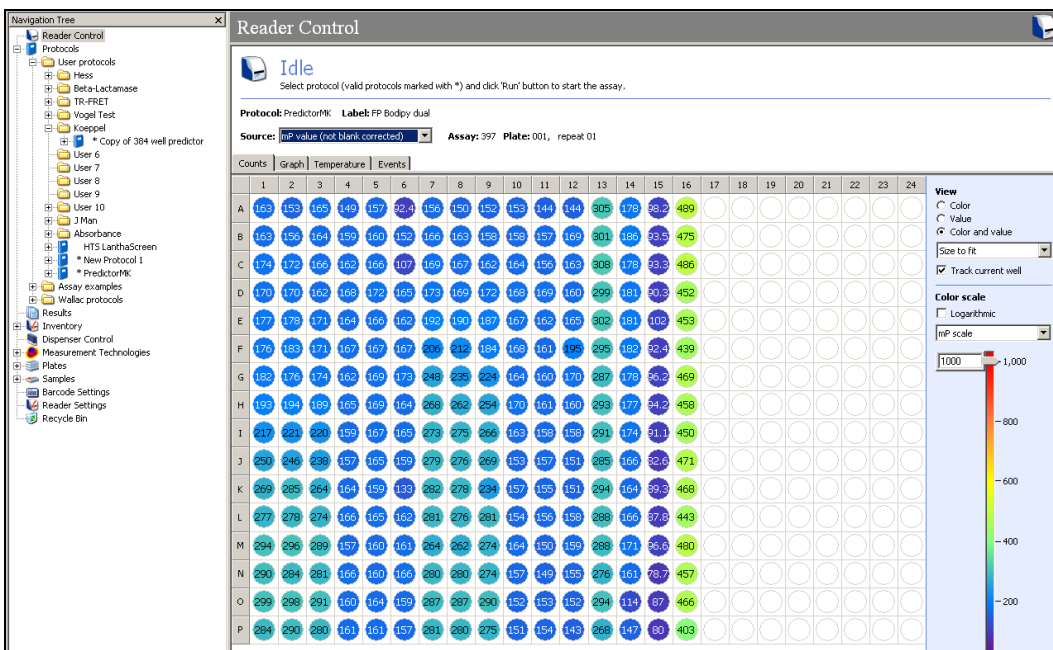
21. When finished, you can retrieve your data by clicking on "Results" in the Navigation Tree just beneath your protocol. When the Results screen pops up, you can open the results by double-clicking on your assay ID (397 in this case).
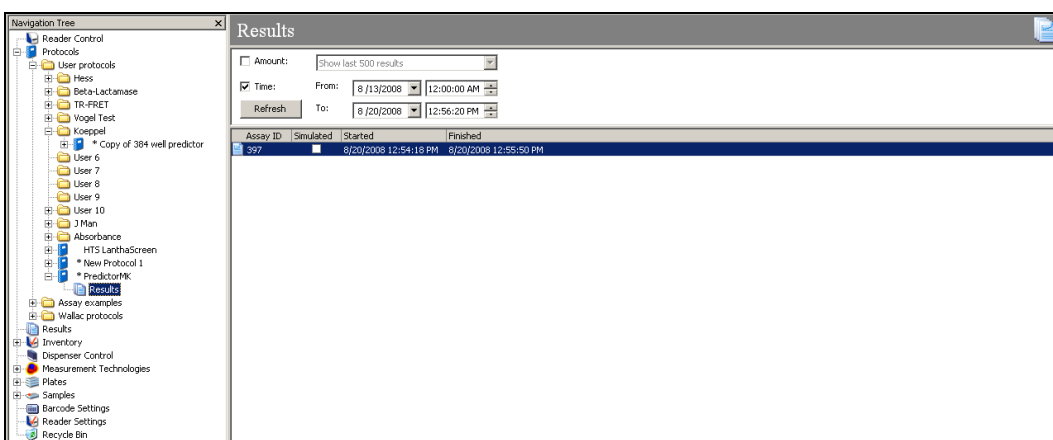
Setup Guide on the PerkinElmer EnVision® Multilabel Reader

22. A new screen will appear. You can view your data here by clicking on the circles just beneath "Calculations" in the main screen, or you can select "Calculations" from the main Navigation Tree and then select "Export" from the toolbar on the top. At this point an Excel file will be created with a prompt for the title and where to save it. **Note**: Here also you can select the button for "Calc 3: mP value (Blank-corrected, blanks on each plate)...." to export blank-corrected values.

Predictor™ Compatible Microplate
Reader Documentation

Version No.:
13 April 12

Page 15 of 19

Setup Guide on the PerkinElmer EnVision® Multilabel Reader
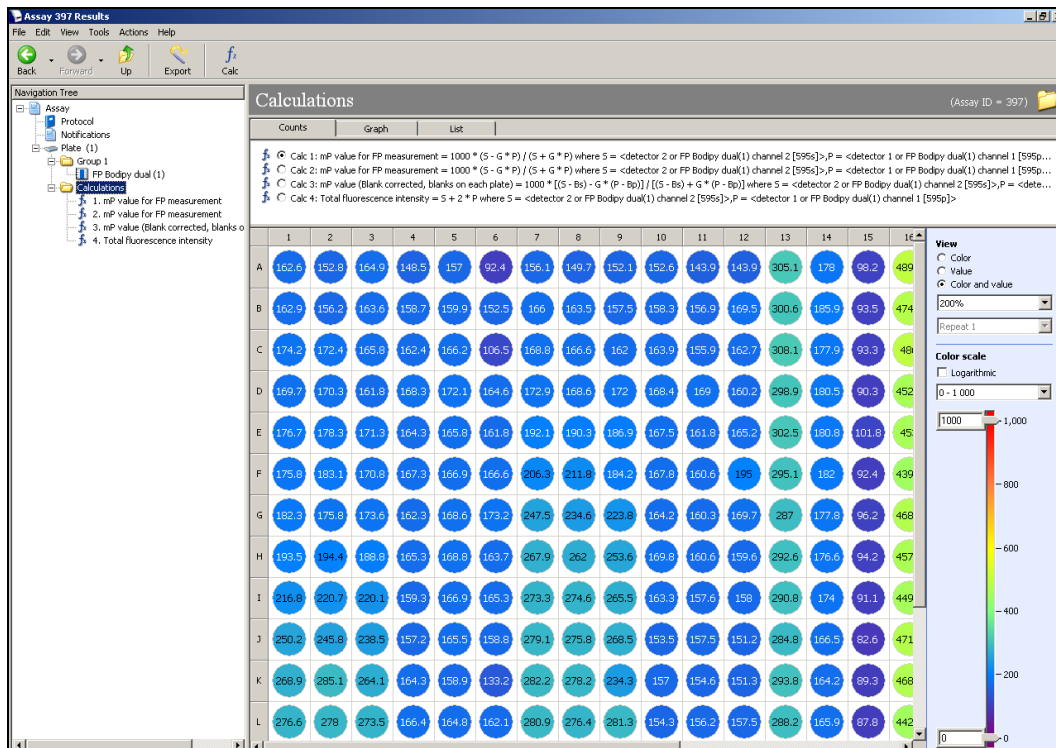
C. Predictor™ hERG FP Assay

Note: The following is a sample assay performed for demonstration purposes. The instrument settings above would be sufficient for any Predictor™ assay or other Invitrogen red FP assay, the information below is provided as representative data. Assays were run in 20 µl in 384-well untreated low-volume polystyrene plates (Corning #3677). In order to demonstrate the correction of Polarization Interference at high concentrations of compound described in the Predictor protocol Section 4.5, we prepared dose-response curves of both astemizole and terfenadine with and without saturating E-4031. Also, all FP data was background-subtracted using wells containing membranes, referred to as Assay Blank, but no tracer.

Note: Background subtraction is not required, but is strongly advised and can increase overall assay window by 10-20%.

1. Thaw all reagents as directed in protocol. Thaw compounds (Astemizole and Terfenadine, 1 mM stocks in DMSO) at room temp.

2. Prepare Predictor™ hERG membrane by trituration to ensure a uniform suspension with no precipitate or aggregates. Sonification or dounce homogenization has also been used.

3. A dilution series of both astemizole and terfenadine was prepared as follows:

3.1 Pre-dilution 1:10. In a 96-well plate, add 40 µl of DMSO to wells B1-H1 and A2-H2 (for Astemizole pre-dilution) and B7-H7 and A8-H8 (for Terfenadine pre-dilution).

3.2 Add 40 µl of 1 mM astemizole to well A1 and 40 µl of 1 mM terfenadine to well A7.

3.3 Prepare Master Dilutions: Transfer 20 µl from well A1 to well A2, mix by pipetting up and down several times, then transfer 20 µl from well A2 to well B1, mix again, then transfer 20 µl from well B1 mixed into well B2, mix again, then transfer 20 µl from well B2 to well C1, repeating this pattern downwards to the bottom of plate (see Figure 1). Repeat this same procedure with terfenadine starting in well A7.

3.4 From well A1, remove 4 µl compound in 100% DMSO and add to well A3 in column 3. From well A2, remove 4 µl compound in 100% DMSO and add to well A4 in column 4. Repeat to bottom of plate, transferring 4 µl aliquots from initial dilution to new column. This step can be simplified by use of a multichannel pipette.

3.5 Repeat for terfenadine, adding 4 µl to columns 9 and 10. When finished, add 96 µl Predictor™ hERG FP Assay Buffer to each new well to create the intermediate dilution series, 4% DSMO.

Setup Guide on the PerkinElmer EnVision® Multilabel Reader

4.   Add 5 µl compound per well to a nontreated Corning 3677 black polystyrene 384-well plate, as follows:

4.1 From well A3 of the 96-well plate (maximum astemizole, conc. 40 µM) transfer 6 replicates of 5 µl compound to wells A1-A6 of the 384 assay plate. We illustrate below showing 3 wells A1 – A3. In the end, column 3 of the 96-well plate will fill the first 6 wells in rows A,C, E, G, I, K, M, and O while column 4 will fill the first 6 wells of rows B, D, F, H, J, L, N, and P. Repeat same procedure was repeated for the next 6 wells in each row on the 384-well assay plate using columns 9 and 10. This step can be simplified by use of a multichannel pipette.
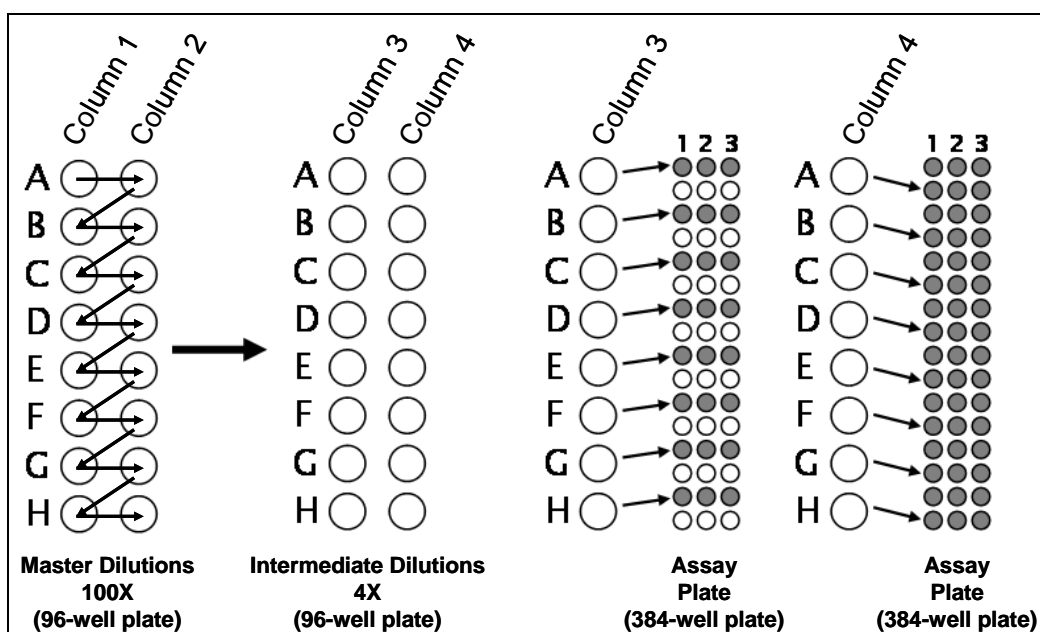

Figure 1: Schematic for Preparation of Compound Dilution. Schematic demonstrates progression of dilutions and final transfer to assay plate. Note in this case 2 compounds were titrated and each compound was finally used in replicates of 6 (3 with and 3 without saturating E-4031).

Predictor™ Compatible Microplate
Reader Documentation

Version No.:
13 April 12

Page 17 of 19

Setup Guide on the PerkinElmer EnVision® Multilabel Reader

5. Once compound titration wells are prepared (columns 1-12 of assay plate), also prepare the following controls:

a. Negative Control, Tracer fully Bound (Column 13, B). Add 5 ul Predictor™ to all wells in Column 13 (to control for DMSO in titration wells). Tracer and membrane will be added later. This control will show full tracer binding .

b. Positive control, Tracer Displaced (Column 14, D). Add 8 µl E-4031 to 192 µl Predictor™ hERG FP Assay Buffer. Add 5 µl per well of this solution to each well of Column 14 of assay plate. Tracer and membrane will be added later. This control will show maximum displacement of tracer from the hERG channels in the membrane prep.

c. Free Tracer Control (Column 15, F). Add 15 µl Predictor™ hERG FP Assay Buffer to each well of column 15. Tracer will be added later. This control will show fully unbound tracer in solution, as well as being used to calibrate the instrument.

d. Assay Blank (Column 16, Blank). Add 10 µl of Predictor™ hERG FP Assay Buffer per well to Column 16. 10 µl membrane will be added later. This control will show background fluorescence and to background subtract the rest of the assay wells prior to calculating FP values.

e. Buffer Blank (not shown). We recommend that Assay Buffer only be used in conjunction with the Free Tracer Control as neither of these preparations contain membrane.

6. Sonicated/triturated membrane split: remove 2 ml Predictor™ membrane and add 40 µl E-4031 to these membranes.

7. Add 10 µl E-4031-containing membrane per well to columns 4-6 (astemizole with E-4031 baseline subtraction) and 10-12 (terfenadine with E-4031 baseline subtraction).

8. Add 10 µl untreated membrane per well to columns 1-3 (astemizole), 7-9 (terfenadine), 13 (Bound Tracer), 14 (Displaced Tracer), and 16 (Blank).

9. Tracer added last: Add 32 µl tracer to 1968 µl Predictor™ hERG FP Assay Buffer and add 5 µl/well of this solution to all wells in Columns 1-15.

Note: Predictor™ is a highly sensitive assay. Furthermore, the baseline correction wells use saturating levels of E-4031. **It is extremely important to ensure that pipetting steps are done carefully and there is no carryover/contamination of neighboring wells in Predictor™.** The order of wells in the plate layout may be changed to best facilitate your pipetting and minimizing carryover if desired, but all the controls above are highly recommended.

10. Shake plate on an orbital plate shaker for 30 seconds, cover with foil, and incubate at least 2 hours before reading.

11. Read plate on plate reader as outlined above.

Figure 2: Schematic of Assay Plate Layout. Predictor™ hERG assay was set up with a dose-response curve of Astemizole and Terfenadine (1:3 dilution series from a starting concentration in the top row of 10 µM) prepared in replicates of 6. From this, 3 replicates were assayed for ability to disrupt Predictor™ Tracer binding and 3 were assayed in saturating levels of E-4031 in order to prepare a baseline correction for each data point.

Setup Guide on the PerkinElmer EnVision® Multilabel Reader
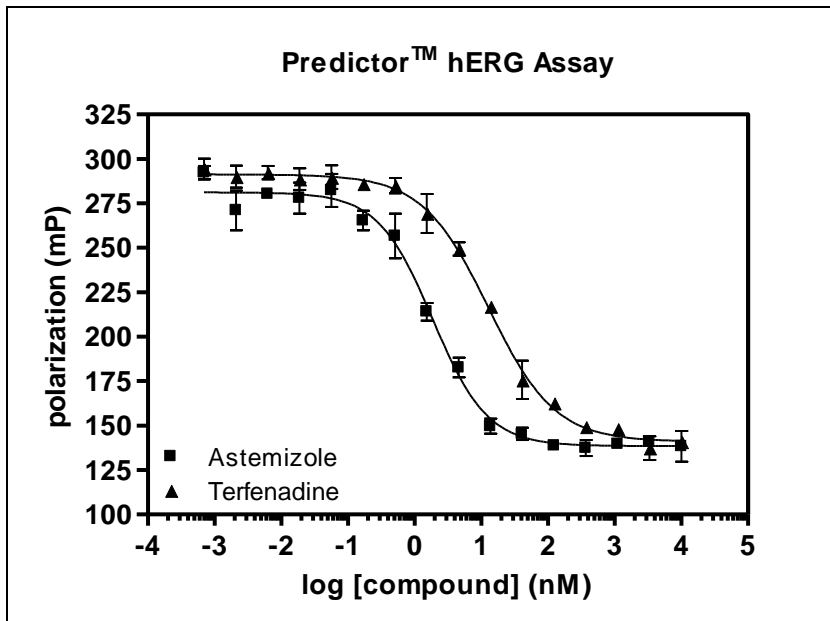
D. Results:

Figure 3: Predictor™ hERG FP Assay. Dose-Response Curves read on the PerkinElmer EnVision® Multilabel Plate Reader using the Predictor™ assay and 1:3 dilution series prepared for Astemizole and Terfenadine from a starting concentration of 10 µM. Curve calculations were baseline-corrected against duplicate dilution series prepared in saturating E-4031.

Table 1. Predictor™ Assay Results on the EnVision®.

	Avg	Std Dev
Free Tracer	56.82	9.35
No Inhibitor	289.43	5.33
30 uM E-4031	138.50	6.89
ΔmP	150.93	
Z'-factor	0.76	
Astemizole IC50	1.87 nM	
Terfenadine IC50	12.89 nM	