Predictor™ hERG Fluorescence Polarization Assay

Frequently Asked Questions – General

What plate reader can be used?

- Most plate readers that can read fluorescence polarization can be used.
- A polarized excitation filter capable of 530 nm excitation, polarized emission filters capable of measuring 580 nm emission, and the appropriate dichroic mirror are required. Please contact your equipment manufacturers for the correct part numbers.
- The exact bandpass and mirror specifications may vary slightly by different instrument vendors. Parts for specific instruments are listed in Table 1.
- Generally, the excitation and emission spectra of the Predictor™ hERG fluorescence polarization tracer are similar to those for tetramethyl rhodamine or TAMRA, and FP instrument settings that have been optimized for those fluorophores will perform well with the Predictor™ tracer.
- CCD-based instruments such as the PerkinElmer ViewLux™ can be used but do require the appropriate flatfield and blank subtraction protocols to be performed (call Invitrogen's tech service if additional help is required).
- Performance will vary from instrument to instrument based on sensitivity and proper optimization.
- Several instruments have been tested by Invitrogen and are summarized in Table 1.

Manufacturer	Instrument	Platform	Window (ΔmP)	Z'-factor
Tecan	Infinite® M1000	Mono	179	0.8
Tecan	Safire^{2}™	Mono	161	0.92
Tecan	Infinite® F500	Filter	167	0.92
BMG LABTECH	PHERAstar/PHERAstar Plus	Filter	170	0.89
MDS Analytical Technologies	SpectraMax™ M5/M5e/FlexStation3	Mono	173	0.48
Perkin Elmer	EnVision	Filter	167	0.79
BioTek	Synergy2/Synergy4	Filter	184	0.7

Table 1: Comparison of Predictor™ hERG FP assay performance on different plate readers. Readers shaded in blue were used on the same day with an identical plate setup. Instruments that provide Z' > 0.5 are recommended for use with the Predictor™ hERG FP assay kit. Using instruments that provide Z' < 0.5 can seriously compromise the interpretation of the data.

What microplates can be used?

- Low volume, non-treated, black, polystyrene 384-well microplates are recommended.
- A number of plates, including 96-well, have been tested and is summarized in Table 2.

Manufacturer	Material	Surface	Part#	Window (ΔmP)	Z'-factor
Greiner	Polystyrene	MediumBind	784076	148	0.91
Corning	Polystyrene	Nontreated	3677	152	0.91
MatriCal	Polypropylene	Nontreated	MP101-1-PP	158	0.87
PerkinElmer	Polystyrene	Nontreated	6008260	154	0.87
Nunc	Polystyrene	Nontreated	264705	151	0.86
Corning	Polystyrene	NBS	3666	126	0.79

Table 2: Comparison of microplates on Predictor™ assay performance

What is the nature of the tracer?

- The Predictor™ hERG Tracer Red was synthesized based on the known pharmacophores that bind the hERG channel at the methanesulfonanilide site in the inner cavity of the channel, near residues Tyr652 and Phe656.
- It has been shown to displace known radioligands that bind that site and has been shown to be displaced by over 30 known hERG channel blockers.
- It has also been shown to block the potassium current conducted by the hERG channel in patch-clamp recordings.

Has the tracer been used in electrophysiological studies?

- The Predictor™ hERG Tracer Red has been shown to block the potassium current conducted by the hERG channel in patch-clamp recordings.

Can I buy the Predictor™ hERG Red Tracer alone?

- No, the Predictor™ hERG Tracer Red and the components of the Predictor™ hERG Fluorescence Polarization Assay have been designed and optimized to work together.
- Attempting to use one of these components individually will likely result in poor assay performance and they are therefore bundled as a kit.

Can I use the Predictor™ hERG Membranes for radioligand binding?

- No, the Predictor™ hERG Membrane are not suitable for radioligand binding.
- These membranes contain extremely high levels of hERG channel protein. Radioligand binding assays would require either abnormally high concentrations of radioligand or severe dilutions of the membrane preparation which tend to have a deleterious effect on the hERG channel conformation and stability.

Does the Predictor™ assay identify blockers missed by RLB?

- Results from beta-testers in the pharmaceutical industry indicate that some compounds missed by radioligand binding assays have been correctly identified by the Predictor™ hERG Fluorescence Polarization Assay.

Does the Predictor™ assay identify blockers missed by patch-clamp?

- Testing is underway to complete a direct comparison of the Predictor™ hERG Fluorescence Polarization Assay to results obtained from automated high-throughput patch-clamping.

Have false-negatives or false-positives been identified?

- Results from beta-testers have identified a small number of compounds that were identified in their internal radioligand binding assays but were not detected in the Predictor™ hERG Fluorescence Polarization Assay.
- Currently, no compound that has been identified by the Predictor™ hERG Fluorescence Polarization Assay as a hERG channel blocker has been found to be a false-positive.
- A set of 41 compounds have been examined internally and compared to IC_{50} values reported or summarized in the published literature.
- These data are tabulated and graphically represented below.

How does the Predictor™ assay compare to radioligand or patch-clamp?

- IC_{50} values determined by the Predictor™ assay correlate extremely well with both radioligand and patch-clamp data.
- These values are compared to several published values in the tables and figures below.
- Tables and figures continue on the following pages

Compound	Assay specific IC_{50} (μM)			
	Radioligand binding		Predictor™	Patch-clamp
	[^{3}H]-astemizole	[^{3}H]-dofetilide		
astemizole	0.004	0.001	0.002	0.001
pimozide	0.019	0.006	0.004	0.018
E-4031	0.075	0.020	0.039	0.048
terfenadine	0.127	0.030	0.021	0.016
dofetilide	0.035	0.040	0.007	0.012
GBR 12909		0.060	0.014	0.007
cisapride	0.158	0.080	0.068	0.020
haloperidol	0.300	0.090	0.174	0.174
droperidol		0.120	0.370	0.032
bepridil	0.782	0.170	0.208	0.550
domperidone		0.220	0.329	0.162
amiodarone	0.752	0.400	0.361	0.700
thioridazine	1.139	0.510	0.536	1.250
mibefradil		0.660	1.318	1.430
verapamil	4.936	0.990	3.802	0.706
propafenone		1.000	0.621	0.440
clozapine		1.200	2.833	0.320
quinidine	13.16	1.310	4.877	0.400
fluoxetine	11.99	2.230	4.083	0.990
imipramine	14.06	4.480	11.38	3.400
flecainide	38.49	4.540	10.09	3.910
pyrilamine		5.000	5.322	1.100
desipramine		5.530	10.96	1.390
sparfloxacin		18.80	86.61	18.00
ketoconazole	19.05	19.500	9.479	4.360
diltiazem		31.20	131.0	17.30
fexofenadine	166.7	32.00	16.13	13.10
spironolactone		45.70	134.5	23.00
grepafloxacin		68.90	>500	50.00
erythromycin	328.5	89.20	>500	152.7
nifedipine			>500	>50
glyburide	318.5		>500	74.00
sertindole			0.023	0.014
flunarizine			0.411	1.950
tamoxifen	0.914		1.131	
amitriptyline	15.55		10.53	10.00
maprotiline			12.17	3.100
papaverine			24.51	7.300

Table 3: Comparison of IC_{50}s determined by the Predictor™ hERG Fluorescence Polarization assay with those determined by patch-clamp and radioligand binding assays. Average fold-differences were found to be 0.5X compared to [^{3}H]-astemizole binding (n=18), 2X compared to [^{3}H]-dofetilide binding (n=28) and 3X compared to patch-clamp recordings (n=33) from heterologous expression systems in mammalian cells. The IC_{50} values are compared by correlation and linear regression analysis (Figure 4) and are further represented by histograms of the IC_{50} FOLD-differences compared to [^{3}H]-dofetilide and patch-clamp in Figure 3 below. Data for [^{3}H]-astemizole binding is from Chiu et al (2004) and data for [^{3}H]-dofetilide is from Diaz et al (2004), which also contains summary references to the patch-clamp data listed here.

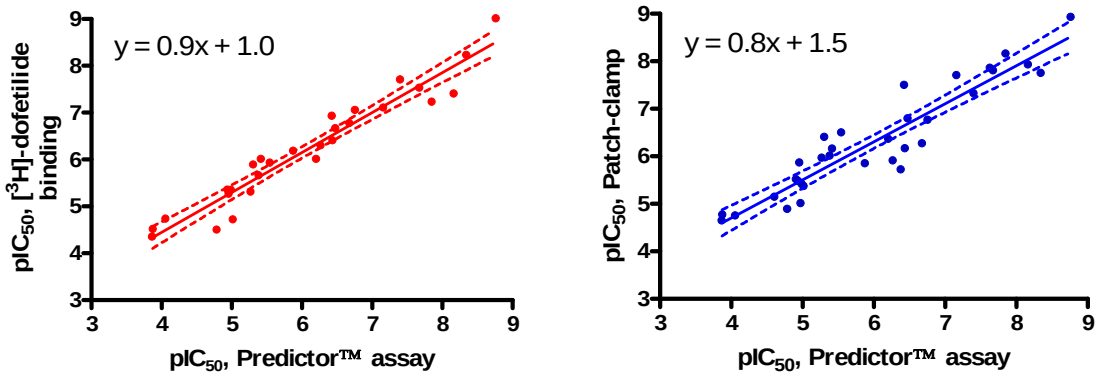

Figure 1: Correlation analysis of the Predictor™ assay to patch-clamp and [^{3}H]-dofetilide radioligand binding assays. Data collected from the literature are plotted as a function of the Predictor™ determined IC_{50} value. Linear regression analysis is plotted on the graphs with the 95% confidence interval shown as dotted lines. Spearman correlation coefficient (r) for the Predictor™ assay vs. [3H]-dofetilide binding was 0.98 (n=28) and for patch-clamp was 0.93 (n=33).