

Predictor™ hERG Fluorescence Polarization Assay Kit performed on the PHERAstar/ PHERAstar Plus



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Introduction

The potential for cardiotoxic side-effects continues to challenge the development of small molecule based therapies. These intolerable side-effects are often precipitated by drug-induced long QT syndrome (LQT), which is often linked to blocking the human ether-a-go-go related gene (hERG) potassium channel.

Although patch-clamp electrophysiology remains the gold standard for determining the interaction of compounds with the function of the hERG channel, radioligand displacement assays have proven to be a cost-effective initial alternative assay for hERG channel liability at early stages of compound development. Speed and cost still suffer however, as radioligand displacement assays remain heterogeneous and depend on the procurement and disposal costs of radioligands.

Because the FDA has recommended that all new drug candidates be tested for blockage of the hERG channel, there is a pressing need for a simple, safe, cost-effective method to triage compounds earlier in the discovery process.

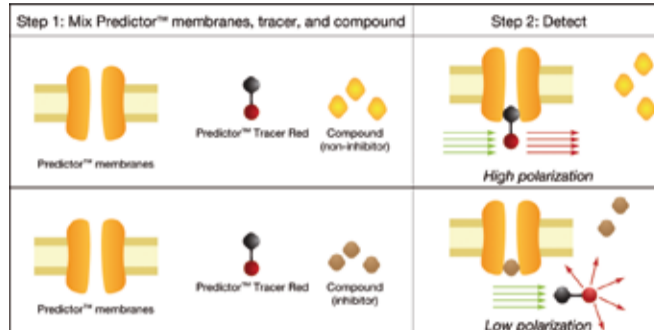


Fig. 1: Schematic of the Predictor™ hERG Fluorescence Polarization Assay

To address this need, the Predictor™ hERG Fluorescence Polarization Assay Kit from Invitrogen has been developed. The assay is based upon the principle of fluorescence polarization (FP), in which a red-shifted fluorescent tracer is displaced from the hERG channel by compounds that bind to the channel. When the tracer is bound to the channel the FP value is high, and when displaced by hERG binding compounds the FP value is low (Figure 1).

This poster describes the performance of the Predictor™ hERG Assay on the BMG LABTECH's PHERAstar/PHERAstar Plus multifunctional HTS microplate reader.

BMG LABTECH's PHERAstar/PHERAstar Plus is a multidetection microplate reader that combines rapid plate reading necessary for high throughput screening (HTS) with enhanced performance and sensitivity needed to read small fluid volumes.



Fig. 2: BMG LABTECH's PHERAstar HTS microplate reader

Results and Discussion

Competitive Binding of Known hERG Blockers

Ten compounds that have been previously shown to block the hERG channel were tested in the Predictor™ hERG Fluorescence Polarization Assay using the BMG LABTECH PHERAstar. The polarization data are shown in Figure 3, with the data summarized and compared with patch-clamp or radioligand displacement data that have been previously reported in Table 1. Non-specific displacement of the tracer from the membrane in the FP assay (observed with some compounds at high concentrations) was corrected by fixing the baseline of the displacement curve to the value seen with saturating levels (30 μ M) of E-4031, a compound for which no non-specific displacement of tracer was observed.

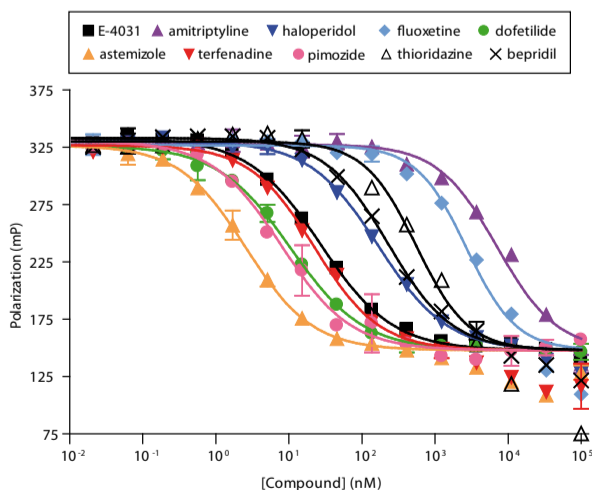


Fig. 3: Competitive binding assay of 10 known hERG blockers using the Predictor™ hERG Fluorescence Polarization Assay

Determination of Assay Z' Value

Assay robustness was assessed by measuring control wells containing either 30 μ M E-4031 (to completely displace tracer from binding to hERG) or no compound. All wells contained 1 % DMSO. The data are shown in Figure 4 and a Z' value of 0.86 was determined.

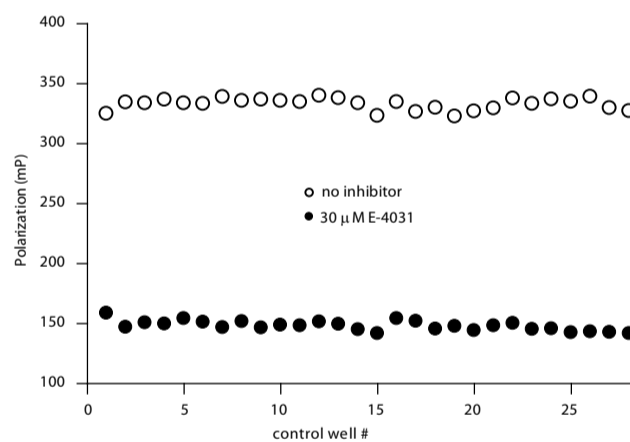


Fig. 4: Z' Control Wells for the Predictor™ hERG Fluorescence Polarization Assay performed on BMG LABTECH's PHERAstar

Although not shown here, the assay is stable at DMSO, methanol, or ethanol concentrations up to 5 %.

Table 1: Comparison of Predictor™ hERG Fluorescence Polarization Assay results with electrophysiology (patch-clamp) or radioligand binding (displacement) data that have been previously reported.

Compound	IC ₅₀ (nM)		
	Predictor™ FP	Patch Clamp	Radioligand
E-4031	27	48	20
amitriptyline	7118	10000	2440
haloperidol	167	174	90
fluoxetine	2687	990	2230
dofetilide	11	12	40
astemizole	3	1.2	1
terfenadine	23	16	30
pimozide	8	18	6
thioridazine	553	1250	510
bepidil	230	550	170

Material and Methods

- PHERAstar microplate reader with Predictor™ FP module (540-20/590-20), BMG LABTECH Offenburg, Germany (Figure 2)
- Black 384 well low volume microplates from Corning
- Predictor™ hERG Fluorescence Polarization Assay Kit from Invitrogen (#PV 5365)

Follow the Predictor™ hERG Fluorescence Polarization Assay Kit instructions. Briefly, a 10 μ L dilution series of the compound was placed in a 384 well plate (in duplicate), followed by 5 μ L additions of both tracer and hERG membrane preparations. The plate could then be read any time between a 1 and 24 hour (or more) incubation.

Conclusion

While assessment of hERG channel function will remain the gold standard to assess hERG channel block, binding assays can be used in order to triage compounds and identify potential liabilities earlier in the process. The recently developed Predictor™ hERG Fluorescence Polarization Assay Kit provides a homogenous assay format that avoids the problems of using radioisotopes to meet this need. In addition, the PHERAstar/PHERAstar Plus multifunctional HTS microplate reader provides one of the fastest and most sensitive platforms to run this FP assay.

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