Using CLARIOstar® fluorescence polarization detection to perform hERG Predictor™ assay

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- Predictor™ hERG Fluorescence Polarization Assay Kits represent an important test of cardiotoxicity
- Using the CLARIOstar® microplate reader you can expect large ΔmP and Z' values, indicative of robust assay performance

Introduction

Acquired long QT syndrome is a drug induced form of cardiac arrhythmia that is characterized by fainting and even sudden death as a result of ventricular fibrillation. The majority of drugs associated with this syndrome cause their effects by interacting with the potassium ion channel Kv11.1, a product of the human Ether-á-go-go-Related-Gene (hERG). Due to the cardiac associated issues it has been recommended that drug candidate interactions with hERG be evaluated in a preclinical environment.

The Predictor™ hERG Fluorescence Polarization Assay Kit was designed to fulfil the need to screen small molecules for the interaction with hERG and thus potential for cardiotoxicity[1]. Since it is based on fluorescence polarization (FP) it can detect hERG interactions without the need for radioactively labelled ligands while exhibiting a high correlation with patch clamp based techniques. Here, we demonstrate the performance of this assay using the CLARIOstar® microplate reader to assess the binding capability of several test compounds on a single microplate.

Assay Principle

The Predictor™ hERG FP Assay uses a tracer which is a fluorescently labelled hERG channel ligand (Fig. 1).

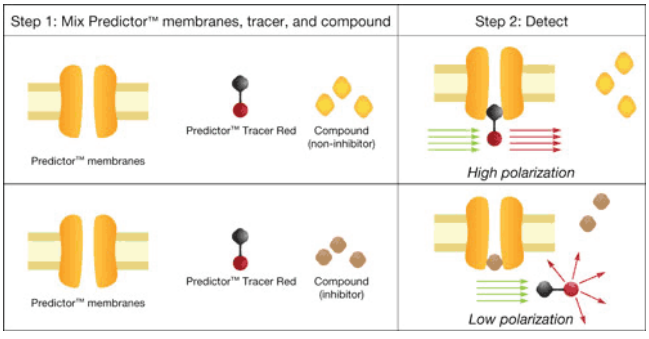

Fig. 1: Predictor hERG Assay Principle. In the absence of a competing compound a high FP value will be observed. Competitors displace the tracer leading to a low fp value.

When the tracer is associated with the membrane bound hERG channel protein the rotation is slowed thus producing a high FP value. A compound that binds to the hERG channel protein will displace the tracer. When the tracer is free in solution it tumbles rapidly and thus exhibits a low FP value.

Materials and Methods

- ☐ Predictor™ hERG Assay Kit (Life Technologies – PV5365)
- ☐ Test compounds (Sigma)
- ☐ Black, 384-well, low volume microplates (Corning – 3677)
- ☐ CLARIOstar multimode microplate reader from BMG LABTECH

Fig. 2: CLARIOstar multimode microplate reader from BMG LABTECH

Kit reagents were thawed and prepared according to the assay instructions and test compounds were dissolved in 100% DMSO. Dilution series for reference and test compounds were prepared using 15 point and 13 point series respectively with 3 fold dilutions. Dilutions were initially prepared at 100X with reference compound dilutions in Assay Buffer and test compound dilutions in 100% DMSO. A 4X intermediate dilution was subsequently prepared in Assay Buffer.

An assay plate was prepared by mixing the appropriate reagents, as indicated in kit instructions, to prepare 20 µL each of Buffer Blank, Assay Blank, Free Tracer Control, Negative Control, Positive Control, E-4031 Titration (reference compound) and Test Compounds Titrations. The plate was then covered, protected from light and incubated for 2 hours at 20-25°C.

Instrument settings

Measurement Method:	Fluorescence Polarization, Endpoint Mode
Filter Settings:	540-20 / LP 565 / 590-20
Settling Time:	0.1
Number of flashes:	200
Focus and gain:	adjusted prior to measurement
Target mP:	set to 50 mP for free tracer

Results and Discussion

A spectral scan was performed on the Predictor™ hERG Tracer Red (Figure 3). This confirmed that the appropriate filters had been selected for detection of this assay.

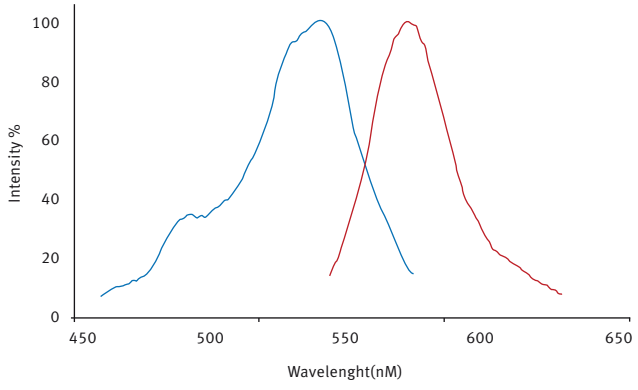

Fig. 3: Excitation and Emission Spectra of Predictor hERG Tracer Red.

The assay performance could be assessed by comparing the positive and negative controls (Fig. 4).

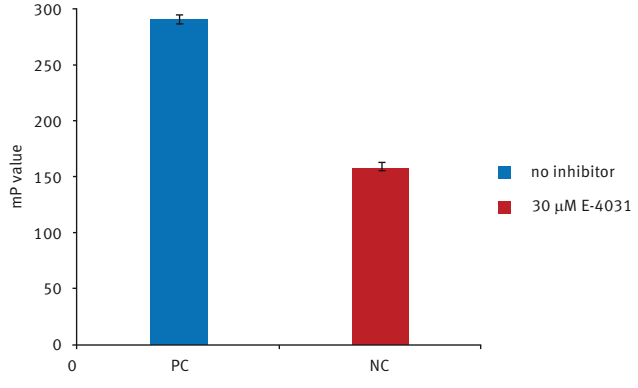

Fig. 4: FP values for positive control (PC) and negative control (NC) of the hERG assay.

The positive control contains 30 µM E-4031 along with tracer and hERG membranes while the negative control does not contain a hERG inhibitor. Comparing these controls show that performing this assay on the CLARIOstar® provides a very good assay window (ΔmP › 130) while a Z´value of 0.752 was achieved.

Assay performance is further exemplified by the reference compound titration curve (Figure 5).

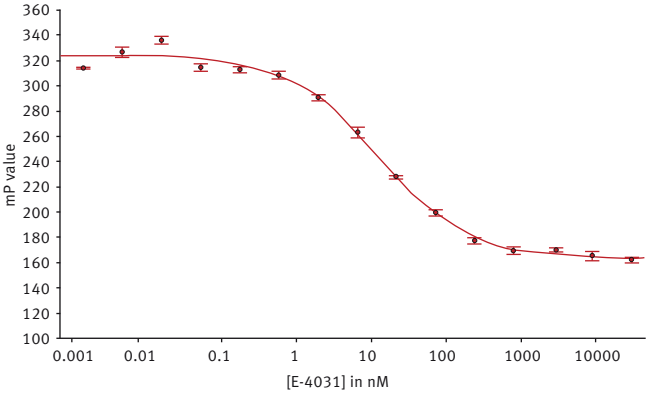

Fig. 5: Reference compound titration curve.

Figure 6 shows the results from 2 test compounds one, HC-067047, was previously reported to interact with the hERG channel (IC_{50} = 370 nM[2]) while the other, ML218, was reported to be a potent T-Type calcium channel inhibitor[3].

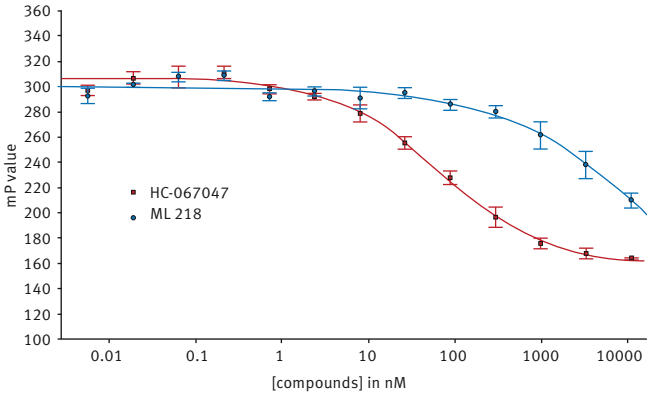

Fig. 6: HC-067047 and ML218 titration curves. Test compound titration curves were prepared with a starting concentration of 11 µM and employed in the hERG assay. The results from HC-067047 treatment conformed to a 4-parameter fit-curve (R^2 = 0.996) with an IC_{50} of 68.4 nM. The data from ML218 did not conform as well to a 4-parameter fit-curve (R^2 = 0.948) and predicted a high IC_{50}.

Conclusion

The CLARIOstar® exhibits robust detection of the Predictor™ hERG assay as exemplified by the Z' values and ΔmP calculated from controls. Furthermore, the reference compound and the test compounds performed as would be predicted based on previous results.

References

1) Piper, D.R., *et al.* (2008) Development of the Predictor hERG Fluorescence Polarization Assay Using a Membrane Protein Enrichment Approach. *Assay Drug Dev. Technol.* **6**, 213-223

2) Everaerts, W., *et al.* (2010) Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. *Proc. Natl. Acad. Sci. USA* **107**, 19084-19089

3) Xiang, Z., *et al.* (2011) The Discovery and Characterization of ML218: A Novel, Centrally Active T-Type Calcium Channel Inhibitor with Robust Effects in STN Neurons and in a Rodent Model of Parkinson's Disease. *ACS Chem. Neurosci.* **2**, 730-742

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