

# Invitrogen's Predictor™ hERG Fluorescence Polarization Assay Using BioTek's Synergy™ 4 Multi-Mode Microplate Reader with Hybrid Technology™

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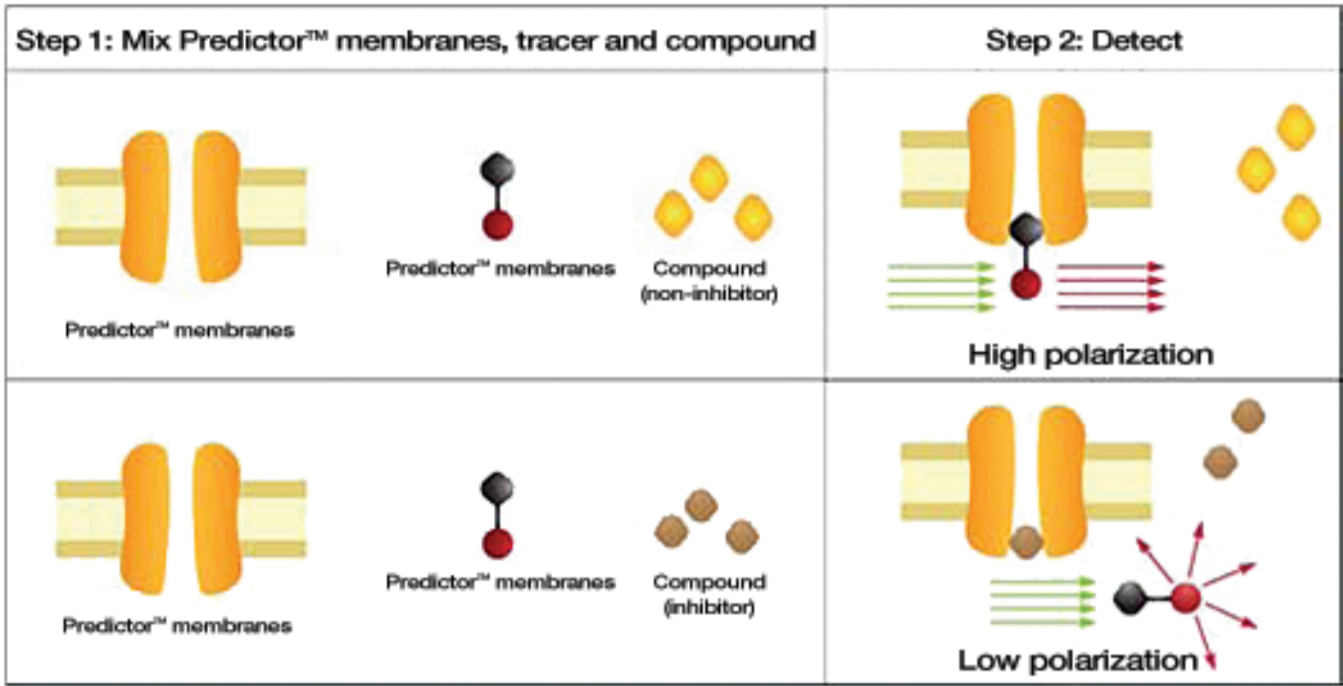
## Assay Workflow Overview for Predictor™ hERG Assay

## hERG Inhibition dose response curves

### Abstract

hERG is one of a family of ion channels shown to be important in the regulation of cardiac rhythm. Although there exist other potential targets for cardiac adverse effects, the vast majority of drugs associated with acquired QT prolongation are known to interact with hERG. Due to the awareness of the potential danger of such QT drugs the regulatory authorities issued recommendations for the establishment of cardiac safety during preclinical drug development. Traditionally, lead compounds in late stage preclinical studies were tested for hERG binding using electrophysiology. These are laborious methods, requiring significant skill in the end-user to perform a successful assay. Furthermore, many researchers wish to test lead compounds for safety earlier in the process of drug development. This requires a higher throughput type of assay. Here we describe results for hERG screening using Invitrogen's fluorescence polarization based Predictor™ assay with a Synergy™ 4 Multi-Mode Microplate Reader with Hybrid Technology™ from BioTek.

### A Complete Solution for High Throughput Drug Safety Test



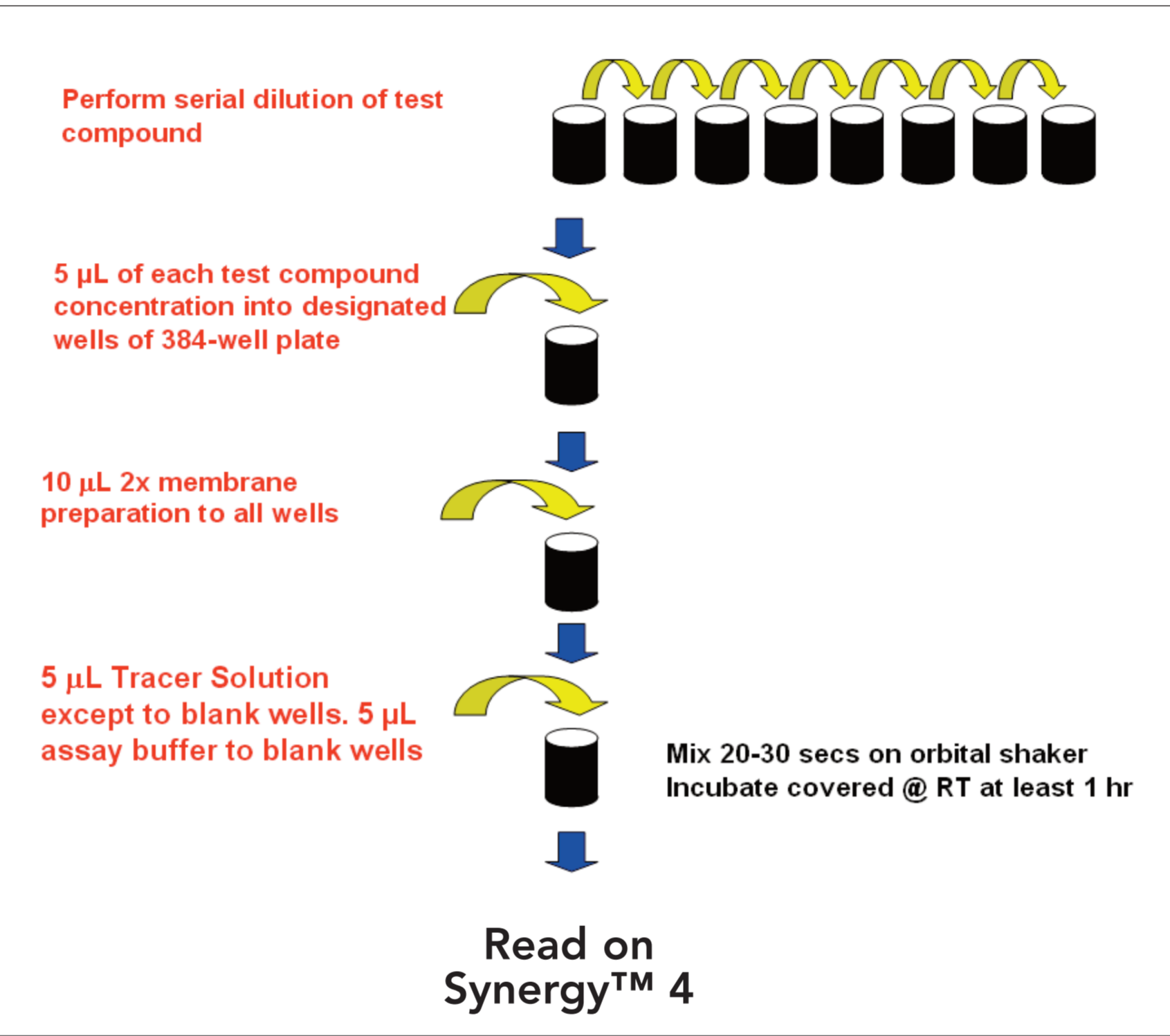
### Predictor™ hERG

- Non-radioactive
- hERG expressed in cell membrane fragments
- Simple “mix and read” Fluorescence Polarization assay

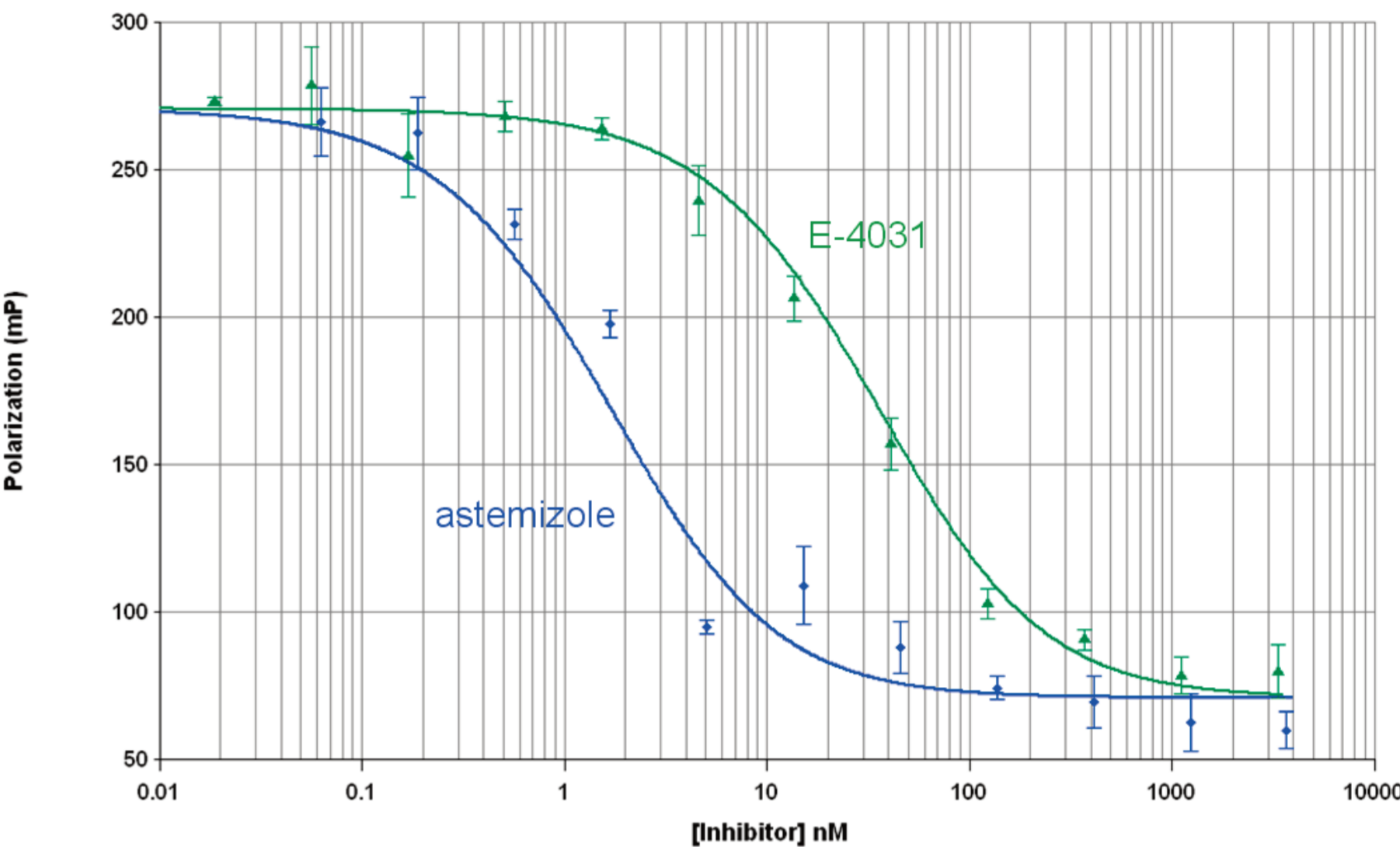
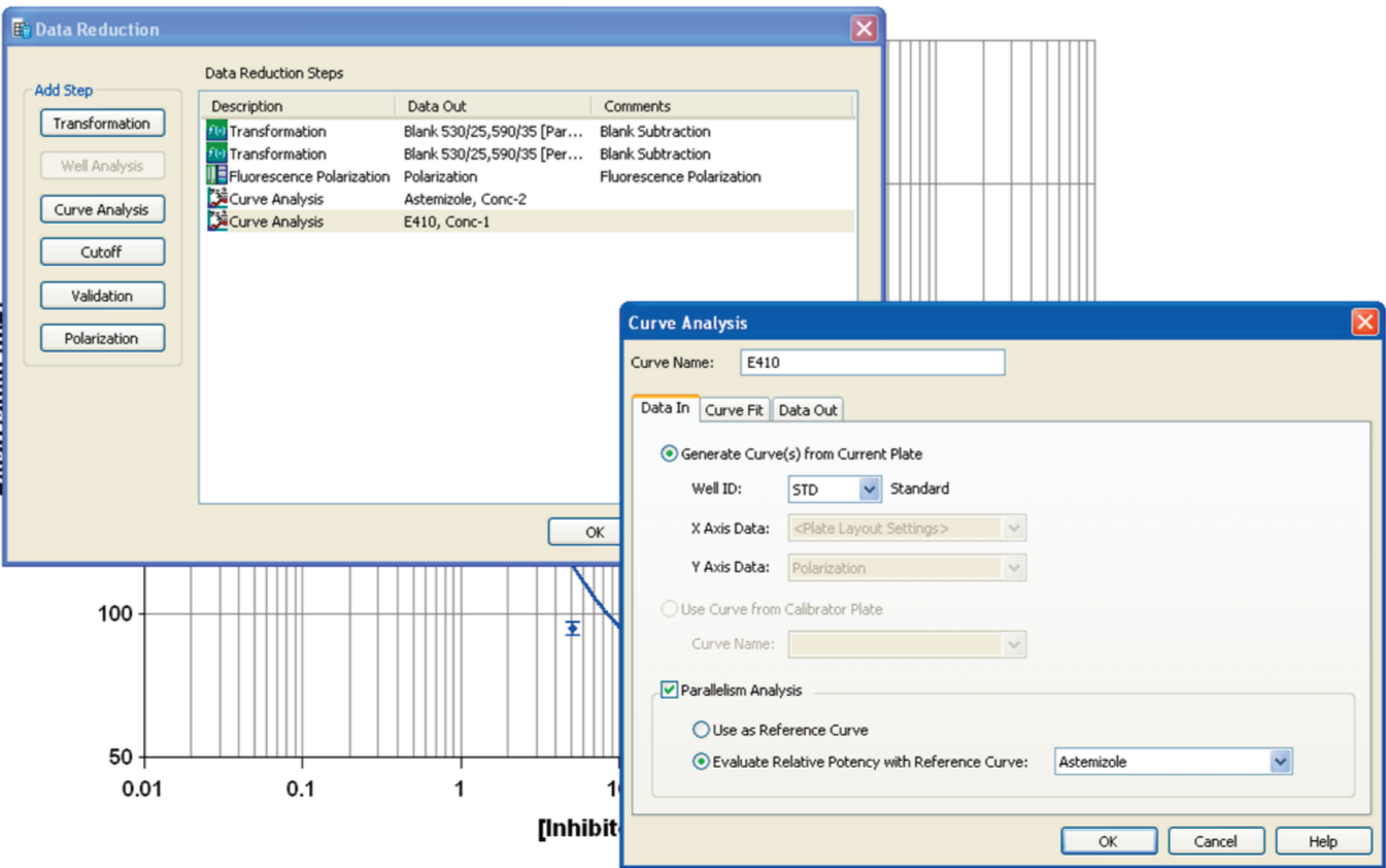


### Synergy™ 4 Multi-Mode Reader with Hybrid Technology™

- Monochromators (Absorbance, Fluorescence)
- Spectral Filters (TR-FRET, FP, Luminescence)



### Gen5 Screen Shots for Parallel Line Analysis



Compound	IC <sub>50</sub> (nM)		
	Patch Clamp	Rad	FP
Astemizole	1.2	1	2
E4031	48	20	40

### Conclusions:

1. Non-radioactive Predictor™ hERG assay simple “mix and read” format.
2. Synergy™ 4 provides suitable assay performance.
3. Gen5 “Parallel Line Analysis” provides IC<sub>50</sub>'s.
4. Pharmacology of known hERG blockers from FP assay agrees with gold standard methods.

- Predictor™ hERG Assay test kits from Invitrogen (Carlsbad, CA).
- Assays were performed in 384-well microplates.
- Fluorescence polarization measurements using Synergy™ 4 Multi-Mode Reader from BioTek Instruments (Winooski VT).
- Measurements were made from the top using the tungsten light source.
- Both parallel and perpendicular fluorescence were measured using the same 530/25-excitation and a 590/35-emission filters.
- 570 nm cut off dichroic mirror.
- PMT sensitivity was set automatically such that the positive control well had a raw fluorescence value for the parallel signal of 50,000 RFU.
- Polarization values were calculated automatically using Gen5™ Data Analysis Software (BioTek Instruments).