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PRODUCT DATASHEET

Precision™ Recombinant hERG Potassium Ion Channel Membrane Preparation

CATALOG NUMBER:	CYL4039	**QUANTITY:**	200 units
LOT NUMBER:	22K1204A	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: The human ether-a-go-go related gene (hERG) is a potassium ion channel which is essential for normal cardiac repolarization. In drug screening models, the hERG K^+ channel has been indicated to inhibit a wide variety of compounds, and its blockage can lead to cardiac QT interval prolongation and life threatening arrhythmias (Murphy *et al*. 2006). Cardiac safety relating to I_{Kr} K^+ channels has become a major concern of regulatory agencies, as hERG channel inhibition has been identified as the firmest link to QT prolongation (Chiu *et al.* 2004). Eurofins hERG membrane preparations are crude membrane preparations made from HEK293 stable recombinant cell lines (Eurofins # CYL3039), which are ideal HTS tools for screening antagonists against the hERG channel. The membrane preparations exhibit a K_d of 2.5 nM for [^{3}H]-Dofetilide. Typically, with 10 µg/well hERG Membrane Prep and 3.0 nM [^{3}H]- Dofetilide, a ≥3-fold signal-to-background ratio is obtained.

APPLICATIONS: Radioligand binding assay

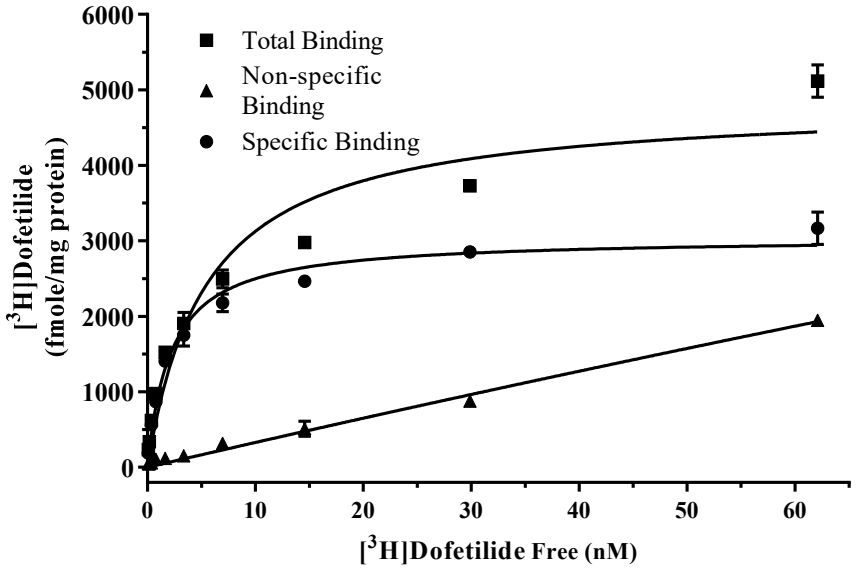

Figure 1. Saturation binding for hERG. 10 µg/well hERG Membrane Preparation was incubated with increasing amount of ^{3}H -labeled Dofetilide in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Dofetilide. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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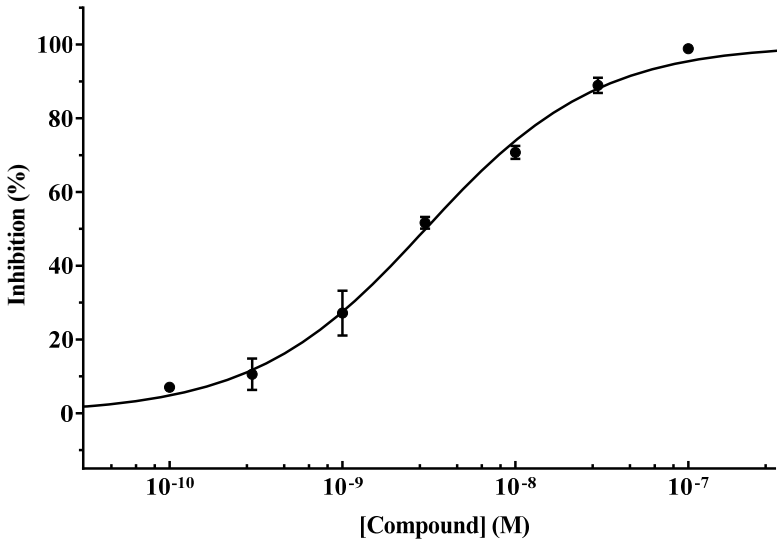

Figure 2. Competition binding for hERG. hERG Membrane Preparation at 10 µg/well was incubated with 3.0 nM [3H]-Dofetilide and increasing concentrations of unlabeled Dofetilide. Typically ≥3-fold signal: background is obtained.

SPECIFICATIONS: 1 unit = 10 µg
B_{max}: 3.0 pmol/mg
K_d: 2.2 nM
Signal:background: ≥3-fold

TRANSFECTION: Human ERG (Accession number U04270)

Species: Human

HOST CELLS: HEK-293

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 10mM HEPES, pH 7.4, 130mM NaCl, 5mM KCl, 0.8mM $MgCl_2$, 1mM NaEGTA, 10mM glucose, 0.1% BSA, filtered and stored at 4°C.

Radioligand: [^{3}H]-Dofefilide (PerkinElmer, NET-1144)

Wash Buffer: 25mM Tris, pH 7.4, 130mM NaCl, 5mM KCl, 0.8mM $CaCl_2$ 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 3-fold signal: background with ^{3}H-labeled Astemizole.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80ºC.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

1. Ackerman MJ *et al.* (1998) The long QT syndrome: ion channel diseases of the heart. *Mayo Clin. Proc.* 73: 250-269.
2. Chiu PJS *et al.* (2004) Validation of a [^{3}H]-Astemizole Binding Assay in HEK293 Cells Expressing hERG K^+ Channels. *J. Pharmacol. Sci.* 95: 311-319.
3. Chouabe C *et al.* (1998) HERG and KvLQT1/IsK, the cardiac K^+ channels involved in long QT syndromes, are targets for calcium channel blockers. *Mol. Pharmacol.* 54: 695-703.
4. Murphy SM *et al.* (2006) Evaluation of functional and binding assays in cells expressing either recombinant or endogenous hERG channel. *J. Pharmacol. Toxicol. Methods 54: 42-55.*
5. Rampe D *et al.* (1997) A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG. *FEBS Lett. 417: 28-32.*