PRECISION™ MEMBRANE PREPARATION RECOMBINANT hERG POTASSIUM ION CHANNEL

(Catalog Number: CYL4039)

CATALOG NUMBER: CYL4039 **QUANTITY:** 200 units (assayed with [^{3}H]-astemizole)

***Note**: this quantity is equivalent to 400 units assayed with [^{125}I]-BeKm.*

LOT NUMBER: RI08030014 **VOLUME/CONCENTRATION:** 2 mL, 1 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). Millipore's hERG membrane preparations are crude membrane preparations made from HEK293 stable recombinant cell lines (Millipore cat. # CYL3039), which are ideal HTS tools for screening antagonists against the hERG channel. The membrane preparations exhibit a K_d of 5.2 nM for [^{3}H]-Astemizole. With 10 μg/well hERG Membrane Prep and 3.0 nM [^{3}H]-Astemizole, a greater than 4-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

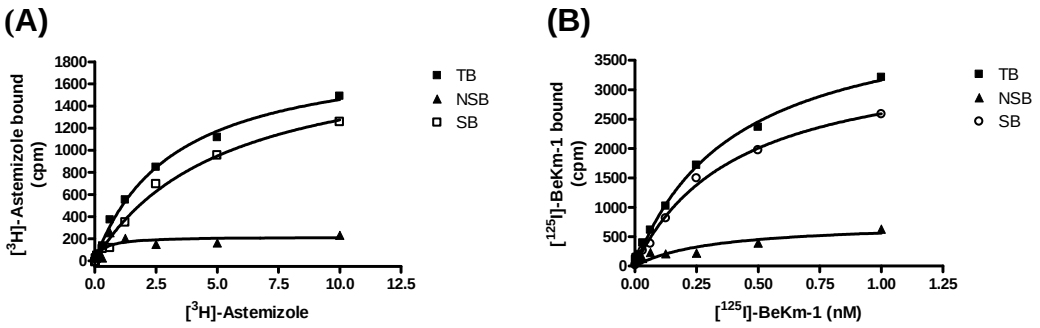

Figure 1. Saturation binding for hERG with [^{3}H]-astemizole and [^{125}I]-BeKm. (A), one unit of hERG membrane preparation was incubated with increasing amount of [^{3}H]-astemizole in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled astemizole. (B), half a unit of hERG Membrane Preparation was incubated with increasing amount of [^{125}I]-BeKm-1 in the absence or presence of 500-fold excess unlabeled BeKm-1. In both curves, specific binding (SB) was determined by subtracting NSB from TB.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com

PRECISION™ MEMBRANE PREPARATION RECOMBINANT hERG POTASSIUM ION CHANNEL

(Catalog Number: CYL4039)

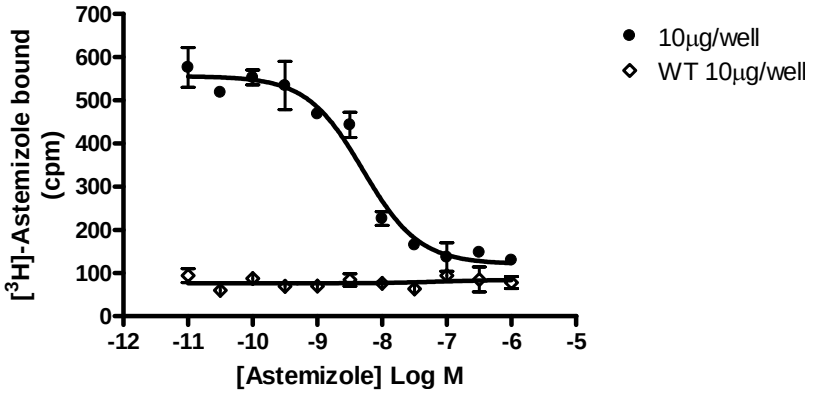

Figure 2. Competition binding for hERG. hERG Membrane Preparation (10 µg/well) or Wild-Type HEK293 membrane preparation was incubated with 3.0 nM [^{3}H]-Astemizole and increasing concentrations of unlabeled Astemizole. More than 4- fold signal: background was obtained.

Table 1. Signal: background and specific binding values obtained in a competition binding assay for the hERG membrane prep.

	10µg/well
Signal: background	4.6
Specific binding (cpm)	435

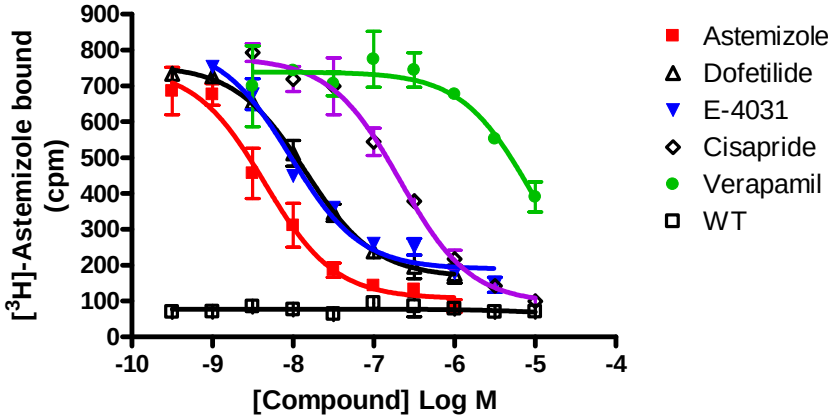

Figure 3. Rank ordering small molecule inhibitors for hERG. hERG Membrane Preparation (10µg/well) was characterized by evaluating the activity for known hERG small molecule inhibitors in a competition binding assay. The membranes were incubated with 3.0 nM [^{3}H]-Astemizole and increasing concentrations of unlabeled compounds to determine sample activity and rank order.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com

Table 2. Rank order comparison of various small molecule hERG inhibitors using [^{3}H]-astemizole binding assay and E-Phys assays:

	Binding Ki (nM) with hERG membrane preps		Electrophysiology IC50 (nM) with hERG cell lines		
	Millipore CYL4039M value	Literature value*	Millipore CYL4039 by IonWorks PPC	Millipore CYL4039 by PatchExpress	Literature value by Patch Clamp
Astemizole	3.4 ± 0.7	3.3 ± 0.7	4.6 ± 0.003	ND	0.5
Dofetilide	5.8 ± 2.4	28 ± 6	ND	ND	15.3 ± 2.5
E-4031	6.1 ± 0.8	58 ± 5	115 ± 0.02	144 ± 44	14
Pimozide	18.8 ± 2.3	14 ± 4	ND	ND	18
Cisapride	104.9 ± 30.9	123 ± 25	75.6 ± 0.02	5 ± 1	44.5 ± 10.6
Haloperidol	241.4 ± 21.4	234 ± 20	ND	ND	63
Risperidone	> 4000	4302 ± 319	ND	ND	167
Verapimil	> 4000	3902 ± 529	1332 ± 120	3100 ± 400	830

* Data obtained from Chiu *et al*., 2004

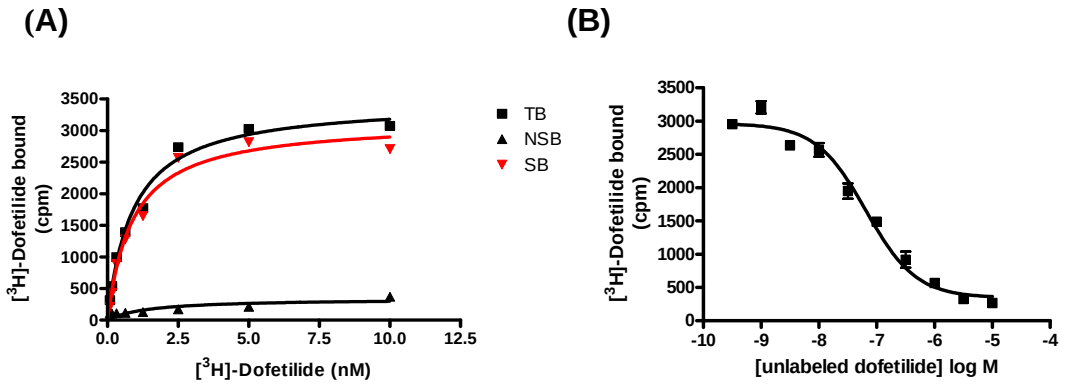

Figure 4. Binding of [^{3}H]-dofetilide to hERG membrane preparation. (A), five units (50 µg) of hERG membrane preparation was incubated with increasing amount of [^{3}H]-Dofetilide (American Radiolabeled Chemicals) in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled Dofetilide. A Kd of 0.9 nM and Bmax of 0.84 pmol/mg were obtained. (B), five units (50 µg) of hERG membrane preparation was incubated with 5 nM [3H]-dofetilide and increasing concentrations of unlabeled dofetilide.

SPECIFICATIONS: 1 unit = 10 µg membrane preparation with [^{3}H]-astemizole
B_{max} with [^{3}H]-astemizole: 6.7 pmol/mg
K_d for [^{3}H]-astemizole: 5.2 nM

Species: Human ERG (Accession number U04270)

HOST CELLS: HEK293

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

PRECISION™ MEMBRANE PREPARATION RECOMBINANT hERG POTASSIUM ION CHANNEL

(Catalog Number: CYL4039)

nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FB 96-well harvest plate (Millipore cat. # MAHF B1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 25 mM Tris, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM $CaCl_2$, 0.1% BSA. Binding reaction is transferred to the filter plate, and washed 6 times (250µL per well per wash) with Wash Buffer. The plate is dried and counted.

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

Radioligand: [^{3}H]-Astemizole (Perkin Elmer # NET-1140)

Wash Buffer: 25 mM Tris, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM $CaCl_2$, 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 4-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 protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at –70°C up to expiration date indicated on the label. Do not freeze and thaw.

REFERENCES:

Ackerman MJ *et al*. (1998) The long QT syndrome: ion channel diseases of the heart. *Mayo Clin. Proc.* 73: 250-269.

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.

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.

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.

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.

Yao JA *et al.* (2005) Estimation of potency of HERG channel blockers: impact of voltage protocol and temperature. *J. Pharmacol. Toxicol. Methods*. 52: 146-153.

For research use only; not for use as a diagnostic.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com