I. Introduction

Catalog Number: M00421
Cell Line Name: HEK293/hERG
Gene Synonyms: ERG, ERG1, HERG
Gene Expressed: GenBank Accession Number NM_000238.2; no expressed tags
Host Cell: HEK293
Quantity: 1 vial (1 ml per vial)
Protein Concentration: 1 mg/ml
Storage Buffer: 50 mM HEPES, 0.1 mM EDTA, 10 % glycerol
Application: Binding assay for human Kv11.1 (hERG) channel
Storage: Store at -80°C

II. Background

hERG (human ether-a-go-go related gene) codes for a protein known as Kv11.1 potassium ion channel. This ion channel mediates the repolarizing IKr current in the cardiac action potential. Suppression of IKr by loss of function mutations in the hERG gene or by untoward drug block can prolong the QT interval and predispose patients to the potentially lethal arrhythmia Torsades de Pointes (TdP). A number of clinically drugs in the market have had the tendency to inhibit hERG, which can increase concomitant risk of sudden death as well as cause other side effects. Therefore, hERG channel is an important antitarget to avoid during drug development.
III. Representative Data

Saturation Binding for hERG Channel

![Graph showing saturation binding](image)

- Total binding
- Specific binding
- NSB

\[ B_{\text{max}} = 2.61 \text{ pmol/mg protein} \]
\[ K_d = 3.57 \text{ nM} \]

**Figure 1** 10 µg of membranes prepared from 293 cells stably expressing hERG channels were incubated with indicated concentrations of \([^3H]\)Dofetilide in the absence (total binding) or presence of 1000-fold excess unlabeled Dofetilide (nonspecific binding, NSB). Binding was terminated by rapid filtration. Specific binding was defined by subtracting NSB from total binding. Data were fit to one-site binding equation using a non-linear regression method.

Competition Binding for hERG Channel

![Graph showing competition binding](image)

- \( K_i = 3.4 \text{ nM} \)
- \( IC_{50} = 7.2 \text{ nM} \)

**Figure 2** 10 µg of membranes prepared from 293 cells stably expressing hERG channels were incubated with indicated concentrations of Astemizole in the presence of 10 nM \([^3H]\)Dofetilide. Binding was terminated by rapid filtration. Data were fit to one-site competition equation using a non-linear regression method.
IV. Brief Competition Binding Protocol

1. Incubated 10 µg membranes with radio labeled ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate. Incubated for 60 min at 37°C.

2. Prior to filtration, coat a GF/C 96-well filter plate with 0.5% polyethyleneimine (PEI) for 30 min at 4°C, then washed the plate with 1 ml/well 50mM HEPES, 0.5% BSA (pH 7.4).

3. Transfer the binding mixtures then to the filter plate. After quick filtration, wash the plate for 3 times (3 ml per well totally) with Wash Buffer.

4. Dry the plate for 0.5 h and then add 50 μl scintillation cocktail (Microscint20). Stay for 1min and count on TopCount NXT for 1 min/well.

5. Binding buffer: 10 mM HEPES, pH7.4, 130 mM NaCl, 60 mM KCl, 0.8 mM MgCl₂, 1 mM NaEGTA, 10 mM glucose, 0.1% BSA, filtered and stored at 4°C

6. Wash Buffer: 10 mM Tris-HCl, pH 7.4, filtered and stored at 4°C.

V. References

