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PRODUCT DATASHEET

ChemiScreen™ β_1 Adrenoceptor Membrane Preparation

CATALOG NUMBER:	HTS104M	**QUANTITY:**	200 units
LOT NUMBER:	SC214104	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

The endogenous catecholamines epinephrine and norepinephrine have profound effects on smooth muscle activity, cardiac function, carbohydrate and fat metabolism, hormone secretion, neurotransmitter release, and central nervous system actions. These activities are mediated by GPCRs belonging to two subfamilies, the α- and β-adrenergic receptors (Bylund *et al.*, 1994). The three members of the β-adrenergic receptor family, β_1, β_2 and β_3, couple to G_s to increase cAMP upon activation. In the heart, the β_1 receptor constitutes 70-80% of the β-adrenergic receptors. Activation of cardiac β-adrenergic receptors acutely increases heart rate, cardiac output, and cardiac automaticity, and chronically increases cardiac myocyte apoptosis. In failing hearts, the β_1 subtype is down regulated and desensitized, probably as a result of increased catecholamine levels. As a result, β-adrenergic receptor antagonists (β blockers) are effective in the treatment of congestive heart failure and arrhythmia (Lohse *et al.*, 2003). β_1 adrenoceptor membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression. Thus, they are ideal HTS tools for screening of antagonists of β_1 adrenoceptor interactions. The membrane preparations exhibit a K_d of 7.0 nM for [^{125}I]-Iodo-(-)-Cyanopindolol (ICYP). With 5 µg/well β_1 Adrenoceptor Membrane Prep and 0.25 nM [^{125}I]-ICYP, a greater than 15-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand Binding Assay

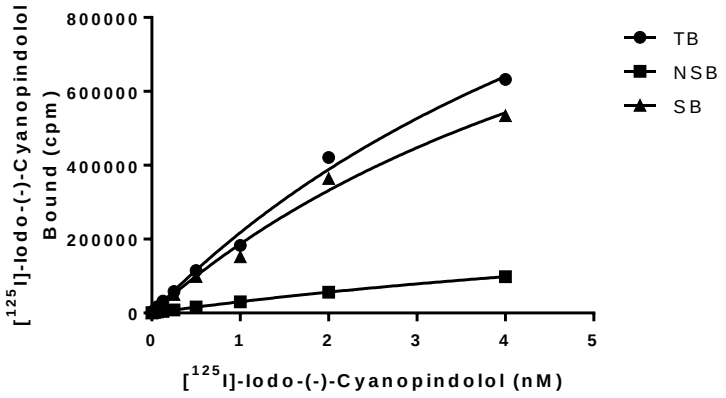

Figure 1. Saturation Binding for β_1 Adrenoceptor. 5 µg/well of β_1 Adrenoceptor Membrane Preparation were incubated with increasing amounts of [^{125}I]-ICYP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled CGP 20712·2HCl. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC214104.

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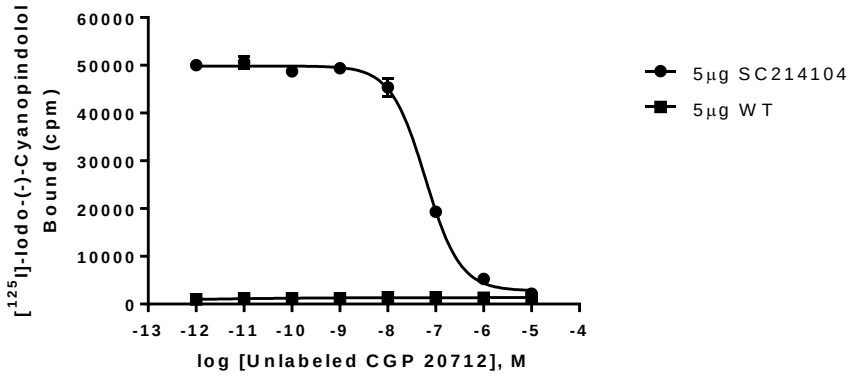

Figure 2. Competition Binding for β_1 Adrenoceptor. 5 µg/well of β_1 Adrenoceptor Membrane Preparation or Wild-Type Chem-1 Membrane Preparation (Catalog # HTS000MC1) were incubated with 0.25 nM [^{125}I]-ICYP and increasing concentrations of unlabeled CGP 20712·2HCl, and more than 15-fold signal:background ratio was obtained. The data are from a representative sample of lot SC214104.

SPECIFICATIONS: 1 unit = 5 µg of membrane preparation
B_{max} for [^{125}I]-Iodo-(-)-Cyanopindolol Binding: 121.6 pmol/mg
K_d [^{125}I]-Iodo-(-)-Cyanopindolol Binding: 7.0 nM
Signal:Background ≥ 15-fold

SPECIES: Full-length human ADRB1 cDNA encoding the β_1 adrenoceptor (Accession number NM_000684)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous β_1 adrenoceptor expression

RECOMMENDED ASSAY CONDITIONS: Membranes were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate, and incubated for 2 h at room temperature. Prior to filtration, an FC 96-well harvest plate was coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4. The binding reactions were transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted for determination of receptor-associated radioligand binding.

Binding Buffer: 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, filtered and stored at 4°C

Radioligand: [^{125}I]-Iodo-(-)-Cyanopindolol (PerkinElmer # NEX189)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM NaCl, 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 a 15-fold signal:background ratio with [^{125}I]-Iodo-(-)-Cyanopindolol at 0.25 nM.

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 1 mg/mL in packaging buffer, dispensed at 1 mL per vial, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Bylund DB *et al.* (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* 46:121-136.

2. Lohse MJ *et al.* (2003) What is the role of β -adrenergic signaling in heart failure? *Circ. Res.* 93:896-906.