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PRODUCT DATASHEET

ChemiScreen™ β_2 Adrenoreceptor Membrane Preparation

CATALOG NUMBER:	HTS073M	**QUANTITY:**	200 units
LOT NUMBER:	22G0603	**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 β -adrenergic receptors, primarily the β_2 subtype, mediate relaxation of smooth muscle in many tissues, and β_2-selective agonists are the preferred drugs for stimulating bronchodilation in the treatment of asthma and chronic obstructive pulmonary disease (Sears and Lotvall, 2005). Activation of the β-adrenergic receptors, primarily the β_1 subtype and to a lesser extent the β_2 subtype, acutely increases heart rate, cardiac output, and cardiac automaticity, and chronically increases cardiac myocyte apoptosis. As a result, β-adrenergic receptor antagonists (β blockers) are effective in the treatment of congestive heart failure and arrhythmia (Feldman *et al.*, 2005). The β_2 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_2 adrenoceptor interactions with (-)Iodocyanopindolol (ICYP). The membrane preparations exhibit Kd of 0.195 nM for [^{3}H]CGP-12177. With 5 µg/well of β_2 Adrenoceptor Membrane Prep and 0.2 nM [^{3}H]CGP-12177, a greater than 10-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand Binding Assay

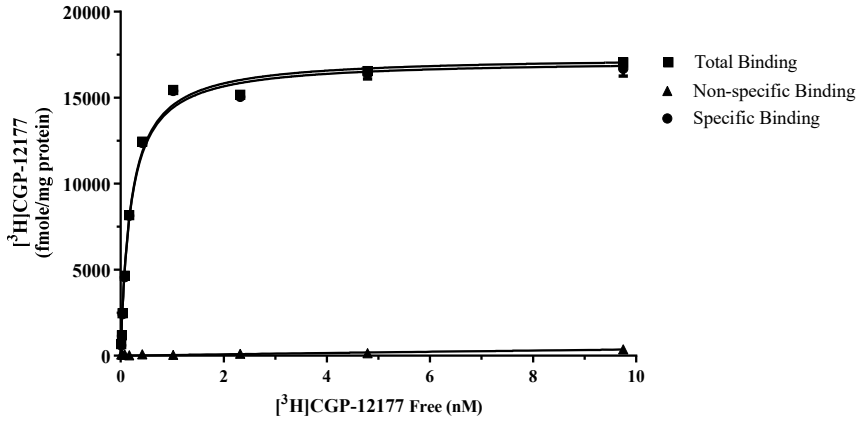

Figure 1. Saturation Binding for β2 Adrenoceptor. 5 µg/well of β2 Adrenoceptor Membrane Preparation was incubated with increasing amounts of [^{3}H]CGP-12177 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled ICI 118,551. Specific binding (SB) was determined by subtracting NSB from TB.

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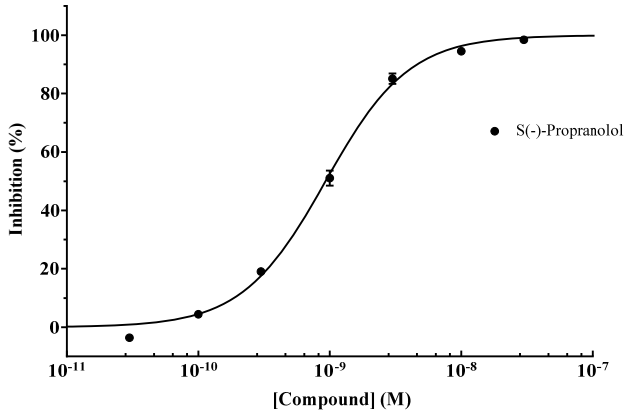

Figure 2. Competition binding for β2 adrenoceptor. 5 µg/well of β2 adrenoceptor Membrane Preparation were incubated with 0.2 nM [^{3}H]CGP-12177 and increasing concentrations of (S)-(-)-Propranolol, and more than a 10-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
Bmax for [^{3}H]CGP-12177 Binding: 17.2 pmol/mg
K_d for [^{3}H]CGP-12177 Binding: 0.195 nM
Signal:Background: >10-fold

Species: Full length human ADRB2 encoding β_2 adrenoceptor (Accession number NM_000024).

HOST CELLS: Chem-2, a suspension mammalian cell line without any endogenous β_2 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 to determine the amount 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: [3H] CGP-12177 (PerkinElmer, NET-1061)

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 40-fold signal:background ratio with [^{125}I]-(-)Iodocyanopindolol at 0.5 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. Feldman DS *et al.* (2005). Mechanisms of Disease: β-adrenergic receptors—alterations in signal transduction and pharmacogenomics in heart failure. *Nat. Clin. Pract. Cardiovasc. Med*. 2:475-83.

3. Sears MR and Lotvall J (2005). Past, present and future— β_2-adrenoceptor agonists in asthma management. *Respir. Med.* 99:152-70.