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PRODUCT DATASHEET

ChemiScreen™ β_3 Adrenergic Receptor Membrane Preparation

CATALOG NUMBER:	HTS159M	**QUANTITY:**	200 units
LOT NUMBER:	SC384996	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND:

The beta adrenergic receptors mediate the effects of endogenous catecholamines, such as epinephrine, by coupling to G_s to stimulate cAMP. Whereas β_1 and β_2 are found predominantly in heart, the β_3 receptor is found primarily in adipose tissue. Activation of adipose β_3 results in lipolysis and thermogenesis. A polymorphism in the human gene for β_3 is associated with weight gain in obese patients (Clement *et al*., 1995). In addition, mice lacking the β_3-adrenoceptor display increased total body fat, particularly on a high fat diet (Revelli *et al*., 1997). These observations indicate that β_3 is a possible target for obesity treatments. The β_3 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_3 adrenoceptor interactions.

APPLICATIONS: Radioligand Binding Assay

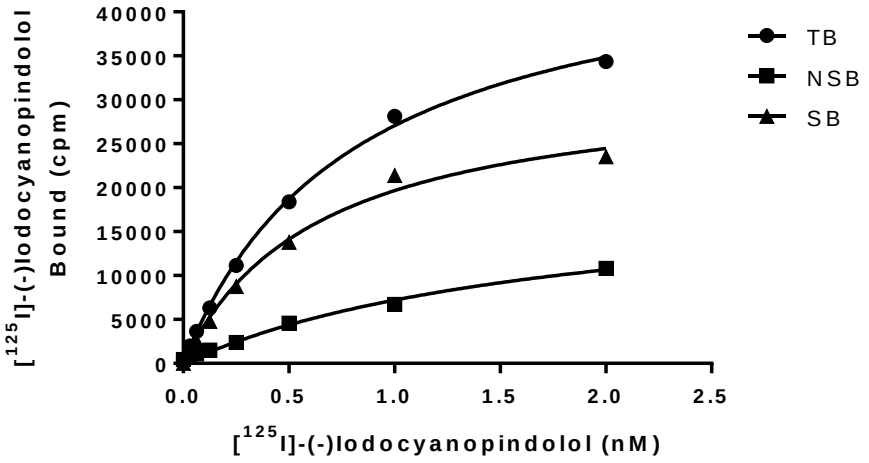

Figure 1. Saturation Binding for β_3 receptor. 10 µg/well β_3 Adrenoceptor Membrane Preparation was incubated with increasing amount of [^{125}I]-(-)ICYP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled SR59230A. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample from lot SC384996.

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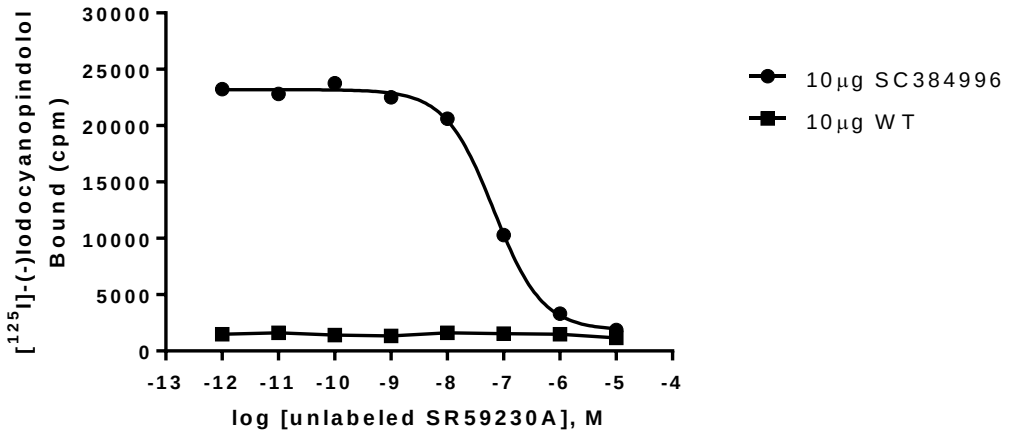

Figure 2. Competition Binding for β_3 adrenoceptor. β_3 adrenoceptor Membrane Preparation (10µg/well) or Wild-Type Chem-1 membrane preparation were incubated with 0.75 nM [^{125}I]-(-)ICYP and increasing concentrations of unlabeled SR59230A, and more than an 8-fold signal:background was obtained. The data are from a representative sample from lot SC384996.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{125}I]-(-)Iodocyanopindolol binding: 1.32 pmol/mg protein
K_d for [^{125}I]-(-)Iodocyanopindolol binding: 0.65 nM
Signal:background: >8-fold

Species: Full-length human ADRB3 cDNA encoding the β_3 adrenoceptor (Accession number NM_000025)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous expression of β_3 adrenergic receptor.

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 non-binding 96-well plate, and incubated for 2 h. Prior to filtration, an FC 96-well harvest plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4. The Binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then dried and counted.

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]-(-)Iodocyanopindolol (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 an 8-fold signal:background ratio with [^{125}I]-(-)Iodocyanopindolol at 0.75 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 the indicated concentration in packaging buffer, 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. Clement K *et al.* (1995). Genetic variation in the beta3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N. Engl. J. Med.* 333:352-354.

2. Revelli JP *et al.* (1997). Targeted gene disruption reveals a leptin-independent role for the mouse beta3-adrenoceptor in the regulation of body composition. *J. Clin. Invest.* 100:1098-1106.