

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN β_3 ADRENOCEPTOR

CATALOG NUMBER:	HTS159M	QUANTITY:	200 units
LOT NUMBER:		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. Millipore's 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. The membrane preparations exhibit a K_d of 0.69 nM for [125 I]-(-)iodocyanopindolol (ICYP). With 10 μ g/well β_3 Adrenoceptor Membrane Prep and 0.75 nM [125 I]-(-)ICYP, a greater than 8-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

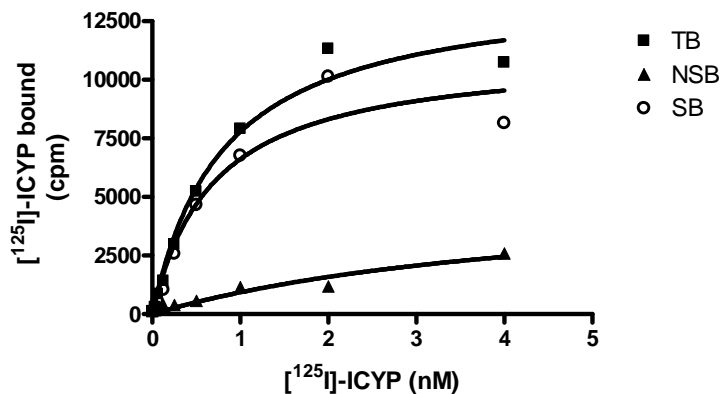


Figure 1. Saturation binding for β_3 receptor. 5 μ g/well β_3 Adrenoceptor Membrane Preparation was incubated with increasing amount of [125 I]-(-)ICYP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled SR59230A. Specific binding (SB) was determined by subtracting NSB from TB.

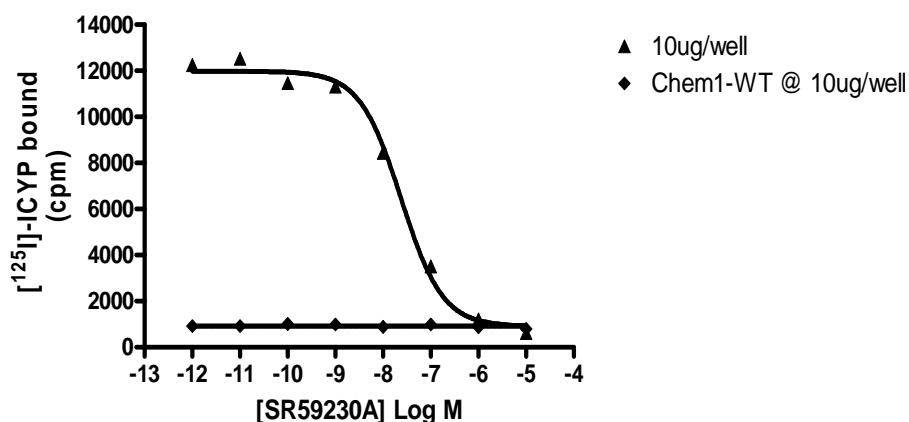


Figure 2. Competition binding for β_3 adrenoceptor. β_3 adrenoceptor Membrane Preparation (10 μ g/well) or Wild-Type Chem-1 membrane preparation (Millipore catalog # HTS000MC1) was incubated with 0.75 nM [125 I]-(-)-ICYP and increasing concentrations of unlabeled SR59230A, and more than 8-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of β_3 adrenoceptor membrane prep.

	10 μ g/well
Signal:background	13.3
Specific binding (cpm)	11074.7

SPECIFICATIONS: 1 unit = 10 μ g membrane preparation

B_{max}: 1.3 pmol/mg

K_d: ~0.69 nM

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

HOST CELLS: Chem-1, a adherent mammalian cell line without any endogenous β_3 adrenoceptor expression.

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 nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM HEPES, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, filtered and stored at 4°C

Radioligand: [125 I]-(-) Iodocyanopindolol (Perkin Elmer # NEX189)

Wash Buffer: 50 mM HEPES, pH 7.4, 500mM NaCl, filtered and stored at 4°C .

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 8-fold signal:background with ¹²⁵I-labeled (-)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: Membranes protein were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

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.

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.

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