upstate | CHEMICON | Linco

THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN β_2 ADRENORECEPTOR RECEPTOR

CATALOG NUMBER:	HTS073M	**QUANTITY:**	200 units
LOT NUMBER:		**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). Millipore's 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 a Kd of 0.51 nM for [^{125}I]-(-)ICYP. With 5 µg/well β_2 Adrenoceptor Membrane Prep and 0.5 nM [^{125}I]-(-)ICYP, a greater than 40-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

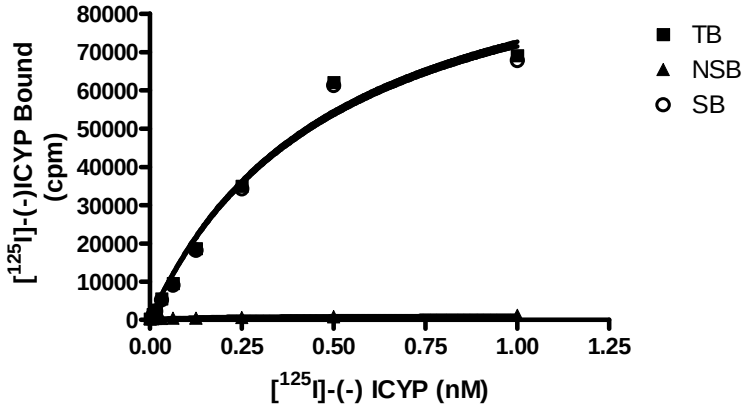

Figure 1. Saturation binding for β2 Adrenoceptor. 5.0 ug/well β2 Adrenoceptor Membrane Preparation was incubated with increasing amount of [^{125}I]-(-)ICYP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled ICI 118,551. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com

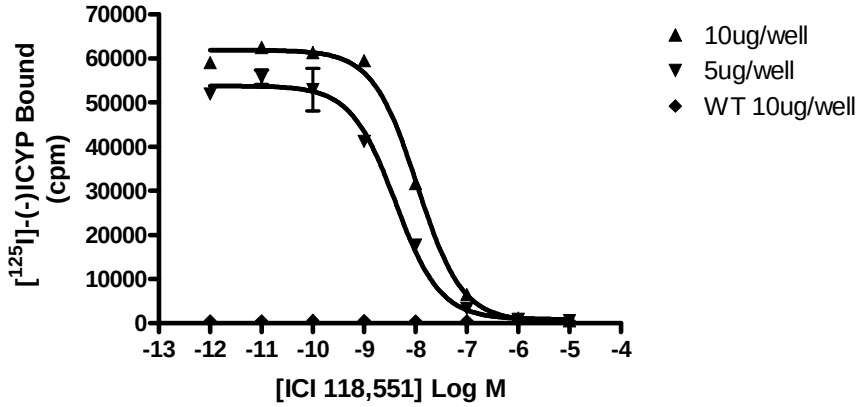

Figure 2. Competition binding for β2 adrenoceptor. β2 adrenoceptor Membrane Preparation (5.0 or 10µg/well) or Wild-Type Chem-2 membrane preparation was incubated with 0.5 nM [^{125}I]-(-)ICYP and increasing concentrations of unlabeled ICI 118,551, and more than 40-fold signal:background was obtained. Sample data from a representative lot.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of β2 adrenoceptor membrane prep.

	5 µg/well	**10 µg/well**
Signal:background	95	103
Specific binding (cpm)	51393	58517

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
Bmax: 8.5 pmol/mg
K_d: 0.51 nM

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 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 $MgCl_2$, 1 mM $CaCl_2$, filtered and stored at 4℃
Radioligand: [^{125}I]-(-) Iodocyanopindolol (Perkin Elmer # NEX189)
Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, filtered and stored at 4℃.

PRESENTATION:

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 40-fold signal:background with ^{125}I-labeled (-)Iodocyanopindolol at 0.5 nM.

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:

Store at –70℃. Product is stable for at least 6 m onths from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

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

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.

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

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION