

PRODUCT DATASHEET
ChemiScreen™ α_{2B} Adrenergic Membrane Preparation

CATALOG NUMBER:	HTS157M	QUANTITY:	200 units
LOT NUMBER:	2106989	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 -adrenoceptors (Bylund *et al.*, 1994). The α_2 adrenergic receptor subfamily members, consisting of α_{2A} , α_{2B} , and α_{2C} , couple primarily to G_i to inhibit cAMP production, and play an important role in regulation of cardiovascular and CNS function. Experiments with α_{2B} -selective agonists and mice lacking α_{2B} demonstrate that α_{2B} plays a role in salt-induced hypotension. Also, the difficulty in breeding homozygous α_{2B} -KO mice indicates the gene may also play an as-yet-unknown role in development or reproduction. (Kable *et al.*, 2000). α_{2B} 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 agonists and antagonists at α_{2B} . The membrane preparations exhibit a K_d of 11.5 nM for [3 H]-Rauwolscine. With 15 nM [3 H]-Rauwolscine, 5 μ g/well α_{2B} Membrane Prep typically yields greater than 30-fold signal-to-background ratio.

APPLICATIONS: Radioligand Binding Assay

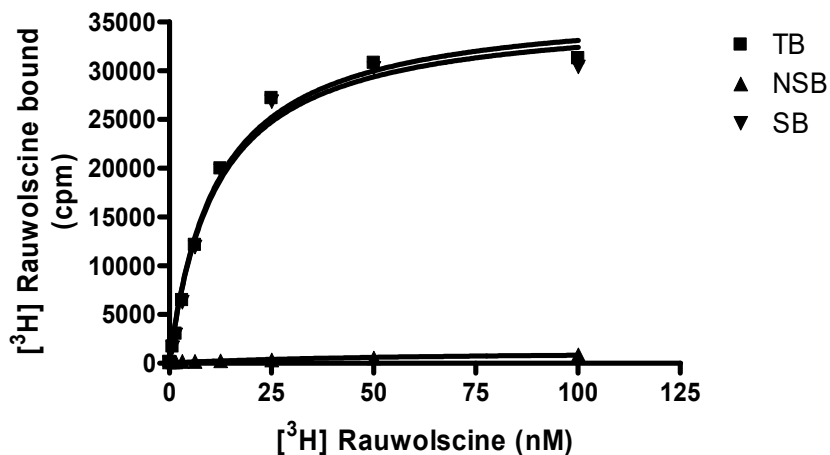


Figure 1. Saturation Binding for α_{2B} . 5 μ g/well α_{2B} Membrane Preparation was incubated with increasing amount of 3 H-labeled Rauwolscine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled rauwolscine. Specific binding (SB) was determined by subtracting NSB from TB.

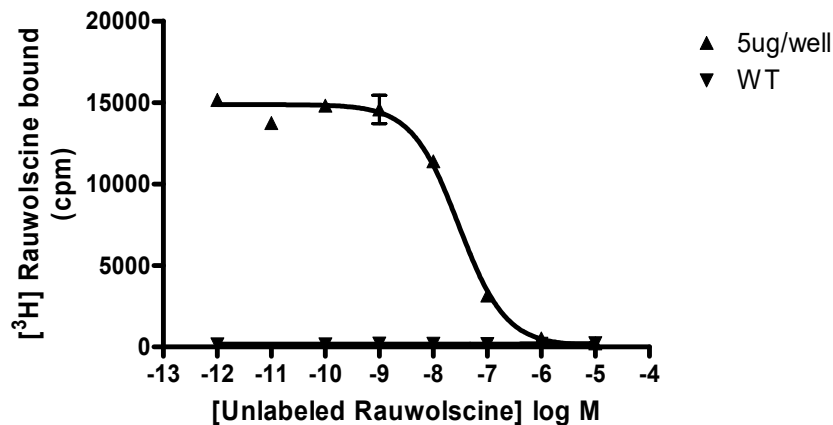


Figure 2. Competition Binding for α_{2B} . 5 mg/well α_{2B} Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 15 nM ^3H -labeled rauwolscine and increasing concentrations of unlabeled rauwolscine. More than 30-fold signal:background was obtained with rauwolscine.

SPECIFICATIONS: 1 unit = 5 μg
 B_{max} for [^3H] rauwolscine binding: 154 pmol/mg protein
 K_d for [^3H] rauwolscine binding: ~ 11.5 nM

TRANSFECTION: Full-length human ADRA2B transcript cDNA encoding α_{2B} (Accession Number: NM_000682)

HOST CELLS: Chem-1, an adherent mammalian cell line with minimum amount of endogenous α_{2B} 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, 0.5% BSA. 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 , 0.2% BSA, filtered and stored at 4°C

Radioligand: [^3H] Rauwolscine (Perkin Elmer#: NET-722)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, 0.1% BSA, 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 30-fold signal:background with ^3H -labeled Rauwolscine at 15 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 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. Kable JW *et al.* (2000). In vivo gene modification elucidates subtype-specific functions of α_2 -adrenergic receptors. *J. Pharmacol. Exp. Ther.* 293:1-7.

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