eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ α_{1B} Adrenergic Membrane Preparation

CATALOG NUMBER:	HTS158M	**QUANTITY:**	200 units
LOT NUMBER:	SC20161011	**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 three members of the α_1 subclass of adrenoceptors, α_{1A}, α_{1B} and α_{1D}, couple to G_q, and promote contraction of vascular and urinary tract smooth muscle, relaxation of intestinal smooth muscle, increased contractile force in the heart, and glycogenolysis and gluconeogenesis in the liver. The different subtypes have overlapping distributions and variably contribute to these effects depending on species and tissue. Overexpression of a constitutively active α_{1B} mutant in the heart of transgenic mice resulted in cardiac hypertrophy with increased heart weight/body weight ratios. Analysis of α_{1B} knock out mice has provided evidence that α_{1B} is a mediator of blood pressure and aortic contractile responses induced by α_1 agonists (Milano *et al.*, 1994). The locomotor and rewarding effects of pysochostimulants and opiates were suppressed in mice lacking α_{1B}-adrenergic receptors (Drouin *et al.* 2002). α_{1B} 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 of α_{1B}. The membrane preparations exhibit a Kd of 0.62 nM for [^{3}H]-Prazosin. With 1 nM [^{3}H]-Prazosin, 5 µg/well of α_{1B} Membrane Prep yields greater than a 5-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

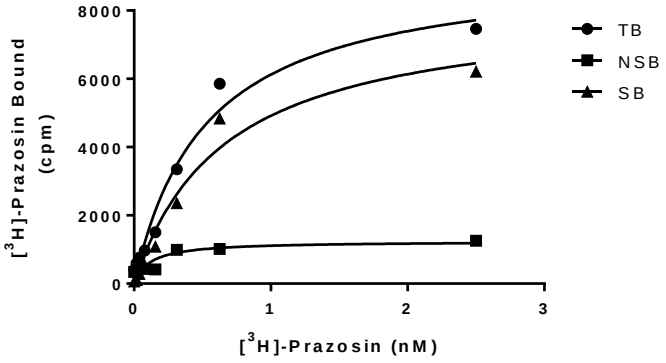

Figure 1. Saturation Binding for α_{1B}. 5 µg/well α_{1B} Membrane Preparation was incubated with increasing amounts of [^{3}H]-Prazosin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Prazosin. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample lot.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

Modified Date: 22DEC14. Modified by: JJP

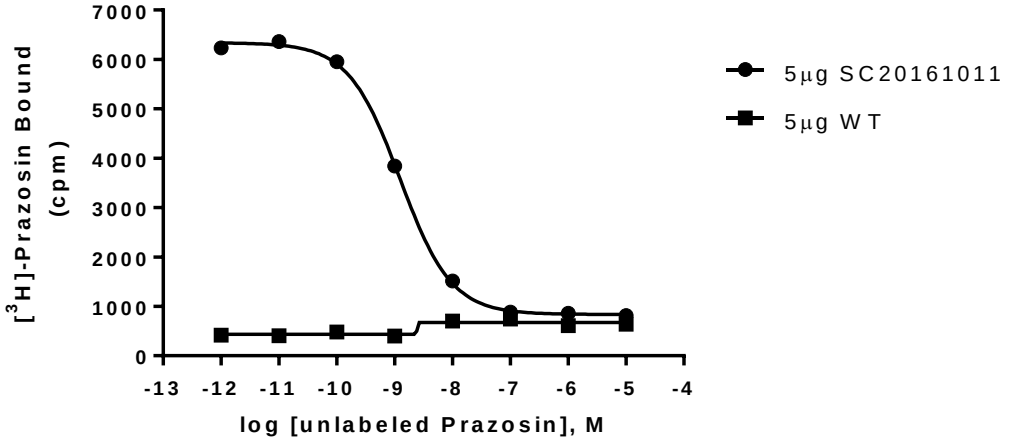

Figure 2. Competition Binding for α_{1B}. 5µg/well α_{1B} Membrane Preparation and Wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 1 nM [^{3}H]-Prazosin and increasing concentrations of unlabeled Prazosin. More than a 5-fold signal:background ratio was obtained. The data are from a representative sample lot.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{3}H]-Prazosin Binding: 15.1 pmol/mg protein
K_d for [^{3}H]-Prazosin Binding: 0.62 nM
Signal:background: >5-fold

TRANSFECTION: Full-length human ADRA1B cDNA encoding α_{1B} adrenergic receptor (Accession Number: NM_000679.3)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous α_{1B} 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 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, and washed with 50 mM Tris, 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 Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, filtered and stored at 4°C

Radioligand: [^{3}H]-Prazosin (PerkinElmer # NET823)

Wash Buffer: 50 mM Tris, pH 7.4, 500 mM 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 a 5-fold signal:background with 1 nM [^{3}H]-Prazosin.

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

2. Cavalli A *et al.* (1997). Decreased blood pressure response in mice deficient of the a_{1B}-AR. *Proc. Natl. Acad. Sci. USA* 94:11589–11594.

3. Milano CA *et al.* (1994). Myocardial expression of a constitutively active α_{1B}-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA* 91:10109-10113.