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PRODUCT DATASHEET

ChemiScreen™ α_{1D} Adrenergic Membrane Preparation

CATALOG NUMBER:	HTS216M	**QUANTITY:**	200 units
LOT NUMBER:	SC20170810	**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. The α_{1D} adrenergic receptor mediates smooth muscle contraction in several tissues. In the vasculature, activation of α_{1D} increases blood pressure (Tanoue *et al*., 2002; Hosoda *et al*., 2005). In the urinary tract, α_{1D} promotes bladder contraction. Antagonists of α_1 receptors are used to treat bladder outlet obstruction, and this effect is thought to be mediated by α_{1D} (Chen *et al*., 2005). The α_{1D} adrenergic receptors has a relatively long N-terminal extracellular domain, and truncation of this domain has been shown to increase expression of the receptor at the cell surface (Pupo *et al*., 2003). The α_{1D} membrane preparations, which contain a version of α_{1D} lacking residues 2-79, 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_{1D}.

APPLICATIONS:

Radioligand Binding Assay

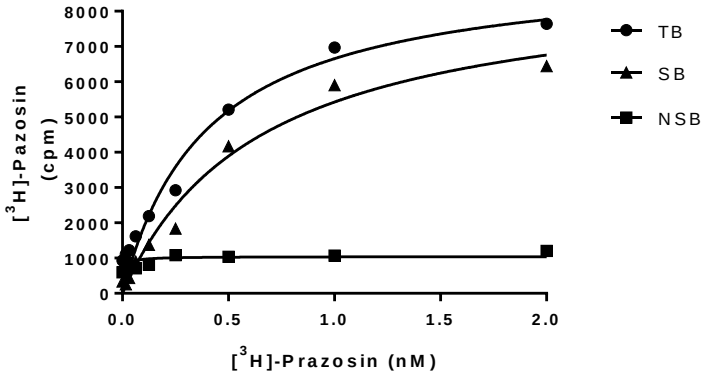

Figure 1. Saturation Binding for α_{1B}. 5 µg/well α_{1B} Membrane Preparation was incubated with increasing amounts of [^{3}H]-Prazosin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Prazosin. Specific binding (SB) was determined by subtracting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

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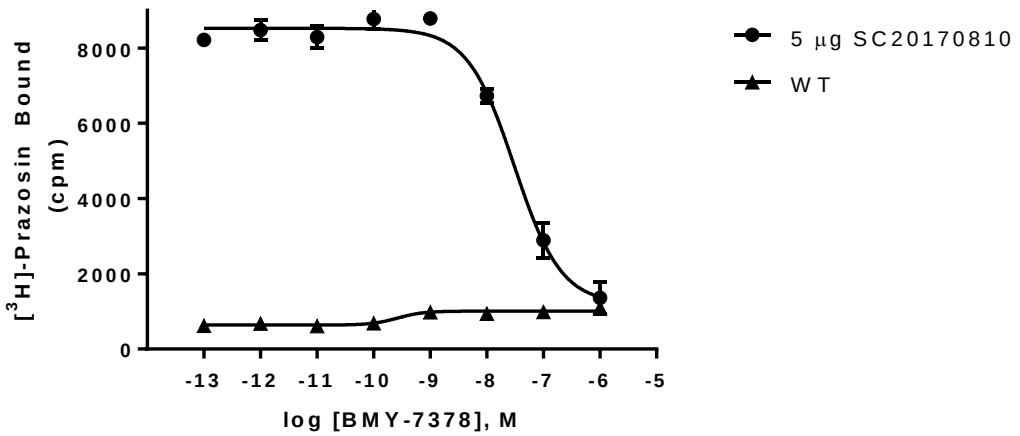

Figure 2. Competition binding for α_{1D}. $\alpha_{1D}(\Delta^{2-79})$ Membrane Preparation (5 and 10 µg/well) and wild-type Chem-1 Membrane Preparation (10 µg/well, catalog # HTS000MC1) were incubated in a 96-well plate with 0.5 nM [^{3}H]-labeled prazosin and increasing concentrations of unlabeled BMY-7378. More than 5-fold signal:background was obtained

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 19.17 pmol/mg
K_d: ~0.65 nM
Signal:background: ≥5-fold

TRANSFECTION: Truncated human ADRA1D cDNA encoding α_{1D} lacking residues 2-79 (based on Accession Number: NM_000678

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous α_{1D} 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: [^{3}H]-Prazosin (PerkinElmer # NET823)

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 5-fold signal:background with ^{3}H labeled prazosin at 0.5 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 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:

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Hosoda C *et al*. (2005) Two α_1-adrenergic receptor subtypes regulating the vasopressor response have differential roles in blood pressure regulation. *Mol. Pharmacol.* 67: 912-922.

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