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PRODUCT DATASHEET

ChemiScreen™ Alpha1A ADRENERGIC RECEPTOR Membrane Preparation

CATALOG NUMBER:	HTS087M	**QUANTITY:**	200 units
LOT NUMBER:	22A0508	**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 α1 subclass of adrenoceptors, α1A, α1B and α1D, couple to Gq, 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 α1A subtype plays a prominent role in urogenital smooth muscle contraction and renal artery contraction (Hrometz *et al*., 1999; Ruffolo and Hieble, 1999). Activation of α1 adrenoceptors also influences cell proliferation; α1A inhibits cell growth by arresting progression at the G1/S transition (Shibata *et al*., 2003). The α1A 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 α1A interactions with prazosin. The membrane preparations exhibit a Kd of 0.9 nM for [^{3}H]-prazosin. With 5 µg/well α1A Membrane Prep and 1 nM [^{3}H]-prazosin, a greater than 5-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

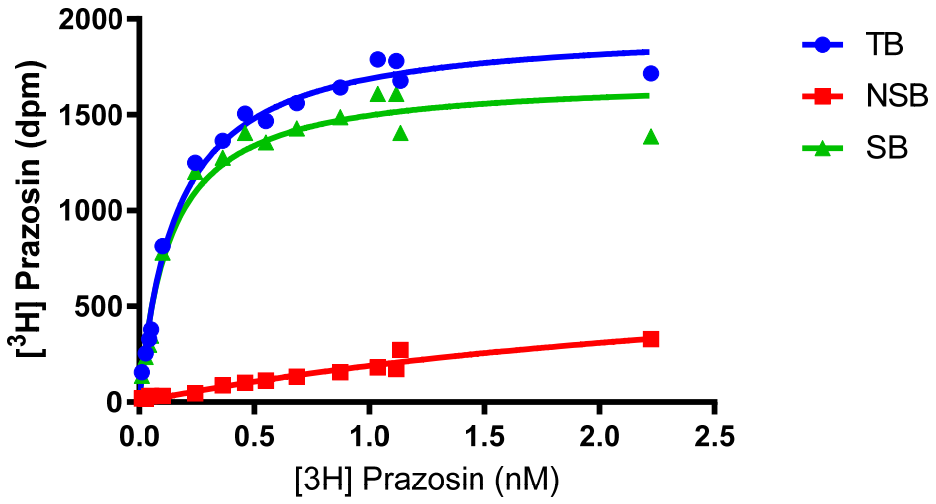

Figure 1. Saturation binding for α1A. 5 µg/well α1A Membrane Preparation was incubated with increasing amount of [^{3}H]-prazosin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled epinephrine. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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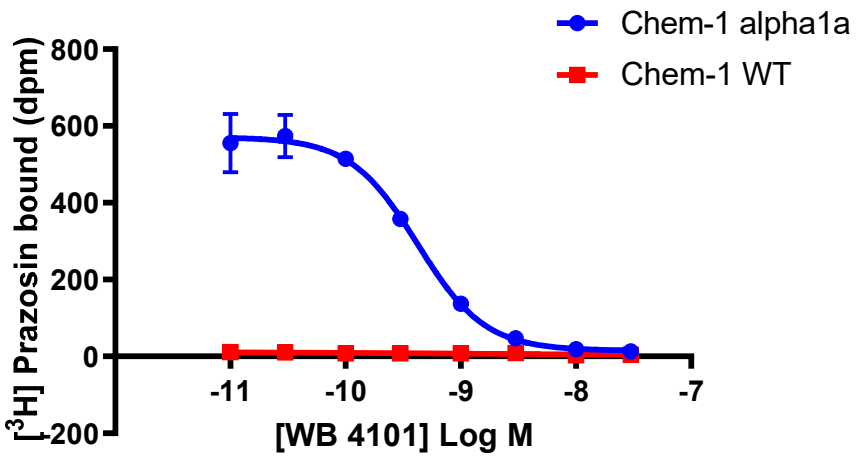

Figure 2. Competition binding for α1A. α1A Membrane Preparation (5 µg/well) or Wild-Type Chem-1 membrane preparation (WT; # HTS000MC1) was incubated with 1 nM [^{3}H]-prazosin and increasing concentrations of unlabeled WB 4101, and more than 5- fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 6.4 pmol/mg protein
K_d: 0.13 nM
Signal:background: >5-fold

TRANSFECTION: Human α1A (Accession number NM_000680)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous α1A 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, a GF/C 96-well filter plate 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 Tris-HCl (pH 7.4), 0.5 mM EDTA, 20 mg/l aprotinin and 0.01% bacitracin, filtered and stored at 4°C

Radioligand: [^{3}H] prazosin (Perkin Elmer # NET823)

Wash Buffer: 50 mM Tris-HCl, 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 1 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris, pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membranes protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80ºC.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

1. Bylund DB *et al.* (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol. Rev. 46: 121-136.
2. Hrometz SL *et al.* (1999) Expression of multiple alpha1-adrenoceptors on vascular smooth muscle: correlation with the regulation of contraction. *J. Pharmacol. Exp. Ther.* 290(1):452-63.
3. Ruffolo JR RR and Hieble JP (1999) Adrenoceptor pharmacology: urogenital applications. *Eur. Urol.* 36 (suppl. 1): 17-22.
4. Shibata K *et al.* (2003) α1-Adrenergic receptor subtypes differentially control the cell cycle of transfected CHO cells through a cAMP-dependent mechanism involving p27Kip1. *J. Biol. Chem.* 278: 672-678.