

**CHEMISCREEN[™] MEMBRANE PREPARATION
RECOMBINANT HUMAN α 1A ADRENERGIC RECEPTOR**

CATALOG NUMBER:	HTS087M	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 -adrenoceptors (Bylund *et al.*, 1994). The three members of the α 1 subclass of adrenoceptors, α 1A, α 1B and α 1D, 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 α 1A subtype plays a prominent role in urogenital smooth muscle contraction and renal artery contraction (Hrometz *et al.*, 1999; Ruffolo and Hieble, 1999). Activation of α 1 adrenoceptors also influences cell proliferation; α 1A inhibits cell growth by arresting progression at the G1/S transition (Shibata *et al.*, 2003). Chemicon's α 1A 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 α 1A interactions with prazosin. The membrane preparations exhibit a Kd of 1.34 nM for [³H]-prazosin. With 5 μ g/well α 1A Membrane Prep and 1 nM [³H]-prazosin, a greater than 7-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay, and GTP γ S binding.

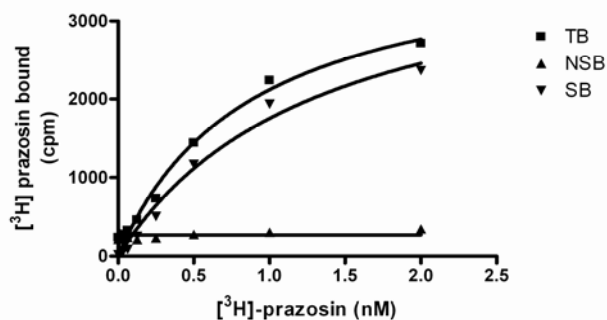


Figure 1. Saturation binding for α 1A. 5 μ g/well α 1A Membrane Preparation was incubated with increasing amount of [³H]-prazosin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled prazosin. Specific binding (SB) was determined by subtracting NSB from TB.

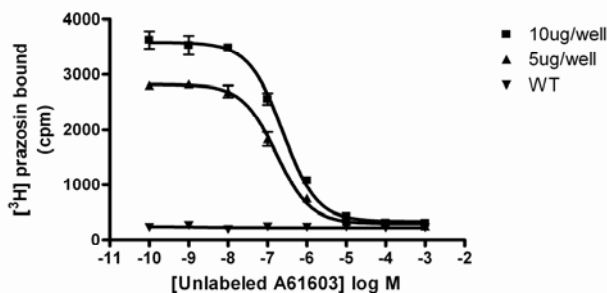


Figure 2. Competition binding for α 1A. α 1A Membrane Preparation (5 μ g/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 1 nM [3 H]-prazosin and increasing concentrations of unlabeled prazosin, and more than 7- fold signal:background was obtained.

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

	10 μ g/well	5 μ g/well
Signal:background	10.7	9.6
Specific binding (cpm)	3244	2530

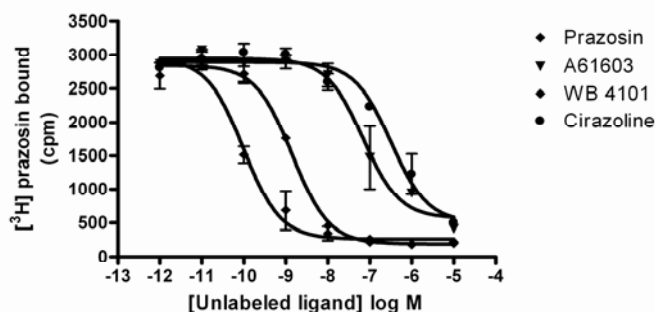


Figure 3. Competition binding for α 1A. α 1A Membrane Preparation (5 μ g/well) was incubated with 1 nM [3 H]-prazosin and increasing concentrations of indicated unlabeled ligands to compare potency of the respective ligands.

SPECIFICATIONS: 1 unit = 5 μ g membrane preparation
Bmax: 11.02 pmol/mg
K_d: 1.34 nM

Species: Human α 1A (Accession number NM_000680)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous α 1A 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 Hepes, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [³H] prazosin (Perkin Elmer # 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 an unit is the amount of membrane that will yield greater than 7-fold signal:background with ³H-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 were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

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

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.

Ruffolo JR RR and Hieble JP (1999) Adrenoceptor pharmacology: urogenital applications. *Eur. Urol.* 36 (suppl. 1): 17-22.

Shibata K *et al.* (2003) α 1-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.

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