MILLIPORE

CHEMICON
now part of Millipore

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN α_{1B} ADRENERGIC RECEPTOR

CATALOG NUMBER:	HTS158M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION PER VIAL:**	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). Millipore's 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 0.8 nM for [^{3}H]-Prazosin. With 1 nM [^{3}H]-Prazosin, 5μg/well α_{1B} Membrane Prep typically yields greater than 5-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay and GTPγS binding.

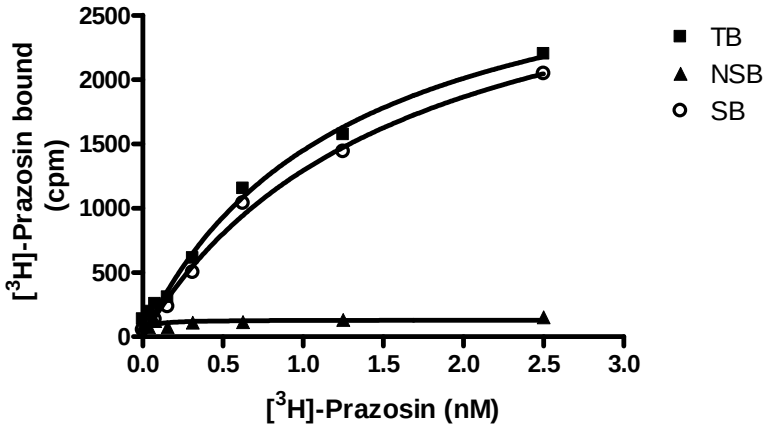

Figure 1. Saturation binding for α_{1B}. 5 μg/well α_{1B} Membrane Preparation was incubated with increasing amount of ^{3}H-labeled Prazosin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Prazosin. Specific binding (SB) was determined by subtracting NSB from TB.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com

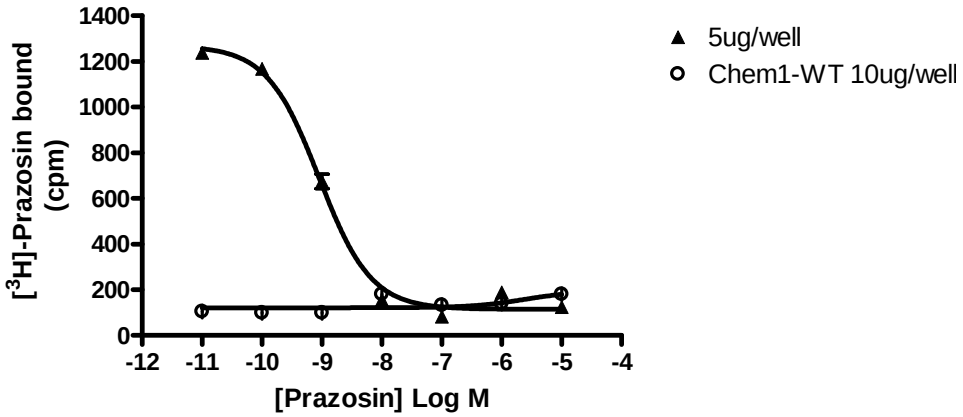

Figure 2. Competition binding for α_{1B}. 5μg/well α_{1B} Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 1 nM ^{3}H-labeled Prazosin and increasing concentrations of unlabeled Prazosin. More than 5-fold signal:background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

Table 1. Signal:background and specific binding values obtained in a competition binding assay with α_{1B} Receptor membrane prep.

	5 μg/well
Signal:background	11.8
Specific binding (cpm)	1171

SPECIFICATIONS: 1 unit = 5 μg
B_{max} for [^{3}H]-Prazosin binding: 12.9 pmol/mg protein
K_d for [^{3}H]-Prazosin binding: ~0.8 nM

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 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 Tris, pH 7.4. 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, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, filtered and stored at 4°C

Radioligand: [^{3}H]-Prazosin. (Perkin Elmer # NET823)

Wash Buffer: 50 mM Tris, 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

MILLIPORE

CHEMICON

now part of Millipore

Prazosin at 1nM

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein were adjusted to 1 mg/ml in 1 ml packaging buffer, rapidly frozen, and stored at -80^{o}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.

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

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.

For research use only; not for use as a diagnostic.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

©2007: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com