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CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN α_{1D} ADRENERGIC RECEPTOR with N-terminal truncation

CATALOG NUMBER:	HTS216M	**QUANTITY:**	200 units
LOT NUMBER:	RI08040047	**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. 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). Millipore's 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}. The membrane preparations exhibit a Kd of 0.4 nM for [^{3}H]-prazosin. With 0.5 nM [^{3}H]-prazosin, 5 µg/well α_{1D} (Δ^{2-79}) Membrane Prep typically yields greater than 5-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

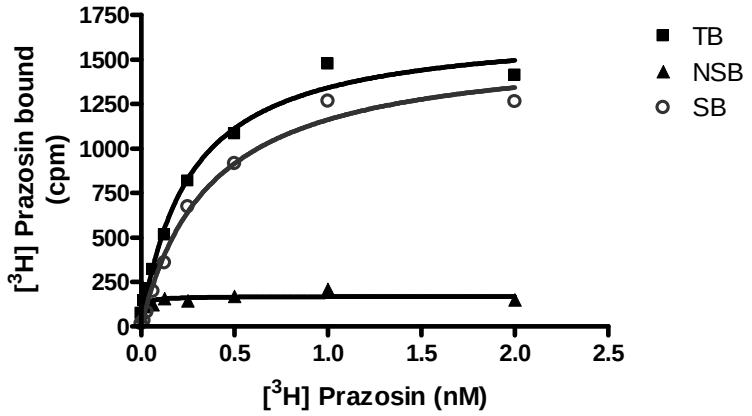

Figure 1. Saturation binding for α_{1D}. 5 µg/well α_{1D} (Δ^{2-79}) Membrane Preparation was incubated with increasing amount of ^{3}H-labeled prazosin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled prazosin. Specific binding (SB) was determined by subtracting NSB from TB.

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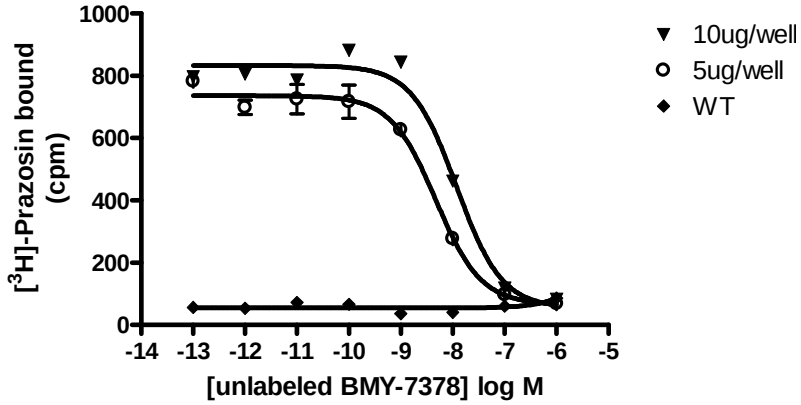

Figure 2. Competition binding for α_{1D}. $\alpha_{1D}(\Delta^{2-79})$ Membrane Preparation (5 and 10 µg/well) and wild-type Chem-1 Membrane Preparation (10 µg/well, Millipore catalog # HTS000MC1) were incubated in a 96-well plate with 0.5 nM [^{3}H]-labeled prazosin and increasing concentrations of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

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of α_{1D} (Δ^{2-79}) receptor membrane prep.

	10 µg/well	5 µg/well
Signal:background	15.4	11.2
Specific binding (cpm)	778.8	670.5

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{3}H]-prazosin binding: 4.23 pmol/mg protein
K_d for [^{3}H]-prazosin binding: ~0.4 nM

TRANSFECTION: Truncated human ADRA1D cDNA encoding α_{1D} lacking residues 2-79 (based on Accession Number: NM_000678; see CODING SEQUENCE below)

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℃

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Radioligand: [^{3}H]-prazosin. (PerkinElmer NET-823)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4℃ .

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: Membranes protein were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Store at –70℃. Product is stable for at least 6 m onths from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

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

Chen Q *et al*. (2005) Function of the lower urinary tract in mice lacking α_{1d}-adrenoceptor. *J. Urol.* 174: 370-374.

Horie K *et al.* (1995) Selectivity of the imidazoline α-adrenoceptor agonists (oxymetazoline and cirazoline) for human cloned α_1-adrenoceptor subtypes. *Br. J. Pharmacol.* 116: 1611-1618.

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.

Pupo AS *et al.* (2003) N-terminal truncation of human α_{1D}-adrenoceptors increases expression of binding sites but not protein. *Eur. J. Pharmacol.* 462: 1-8.

Tanoue A *et al.* (2002) The α_{1D}-adrenergic receptor directly regulates arterial blood pressure via vasoconstriction. *J. Clin. Invest.* 109: 765-775.

CODING SEQUENCE:

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  1 - ATG ACT TTC CGC GAT CTC CTG AGC GTC AGT TTC GAG GGA CCC CGC CCG GAC AGC AGC GCA GGG GGC TCC AGC - 72
  1 -  M   T   F   R   D   L   L   S   V   S   F   E   G   P   R   P   D   S   S   A   G   G   S   S - 24

 73 - GCG GGC GGC GGC GGG GGC AGC GCG GGC GGC GCG GCC CCC TCG GAG GGC CCG GCG GTG GGC GGC GTG CCG GGG - 144
 25 -  A   G   G   G   G   G   S   A   G   G   A   A   P   S   E   G   P   A   V   G   G   V   P   G - 48

145 - GGC GCG GGC GGC GGC GGC GGC GTG GTG GGC GCA GGC AGC GGC GAG GAC AAC CGG AGC TCC GCG GGG GAG CCG - 216
 49 -  G   A   G   G   G   G   G   V   V   G   A   G   S   G   E   D   N   R   S   S   A   G   E   P - 72

217 - GGG AGC GCG GGC GCG GGC GGC GAC GTG AAT GGC ACG GCG GCC GTC GGG GGA CTG GTG GTG AGC GCG CAG GGC - 288
 73 -  G   S   A   G   A   G   G   D   V   N   G   T   A   A   V   G   G   L   V   V   S   A   Q   G - 96

289 - GTG GGC GTG GGC GTC TTC CTG GCA GCC TTC ATC CTT ATG GCC GTG GCA GGT AAC CTG CTT GTC ATC CTC TCA - 360
 97 -  V   G   V   G   V   F   L   A   A   F   I   L   M   A   V   A   G   N   L   L   V   I   L   S - 120

361 - GTG GCC TGC AAC CGC CAC CTG CAG ACC GTC ACC AAC TAT TTC ATC GTG AAC CTG GCC GTG GCC GAC CTG CTG - 432
121 -  V   A   C   N   R   H   L   Q   T   V   T   N   Y   F   I   V   N   L   A   V   A   D   L   L - 144

433 - CTG AGC GCC ACC GTA CTG CCC TTC TCG GCC ACC ATG GAG GTT CTG GGC TTC TGG GCC TTT GGC CGC GCC TTC - 504
145 -  L   S   A   T   V   L   P   F   S   A   T   M   E   V   L   G   F   W   A   F   G   R   A   F - 168

505 - TGC GAC GTA TGG GCC GCC GTG GAC GTG CTG TGC TGC ACG GCC TCC ATC CTC AGC CTC TGC ACC ATC TCC GTG - 576
169 -  C   D   V   W   A   A   V   D   V   L   C   C   T   A   S   I   L   S   L   C   T   I   S   V - 192

577 - GAC CGG TAC GTG GGC GTG CGC CAC TCA CTC AAG TAC CCA GCC ATC ATG ACC GAG CGC AAG GCG GCC GCC ATC - 648
193 -  D   R   Y   V   G   V   R   H   S   L   K   Y   P   A   I   M   T   E   R   K   A   A   A   I - 216

649 - CTG GCC CTG CTC TGG GTC GTA GCC CTG GTG GTG TCC GTA GGG CCC CTG CTG GGC TGG AAG GAG CCC GTG CCC - 720
217 -  L   A   L   L   W   V   V   A   L   V   V   S   V   G   P   L   L   G   W   K   E   P   V   P - 240

721 - CCT GAC GAG CGC TTC TGC GGT ATC ACC GAG GAG GCG GGC TAC GCT GTC TTC TCC TCC GTG TGC TCC TTC TAC - 792
241 -  P   D   E   R   F   C   G   I   T   E   E   A   G   Y   A   V   F   S   S   V   C   S   F   Y - 264
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 793 - CTG CCC ATG GCG GTC ATC GTG GTC ATG TAC TGC CGC GTG TAC GTG GTC GCG CGC AGC ACC ACG CGC AGC CTC  - 864
 265 -  L   P   M   A   V   I   V   V   M   Y   C   R   V   Y   V   V   A   R   S   T   T   R   S   L  - 288

 865 - GAG GCG GGC GTC AAG CGC GAG CGA GGC AAG GCC TCC GAG GTG GTG CTG CGC ATC CAC TGT CGC GGC GCG GCC  - 936
 289 -  E   A   G   V   K   R   E   R   G   K   A   S   E   V   V   L   R   I   H   C   R   G   A   A  - 312

 937 - ACG GGC GCC GAC GGG GCA CAC GGC ATG CGC AGC GCC AAG GGC CAC ACC TTC CGC AGC TCG CTC TCC GTG CGC  - 1008
 313 -  T   G   A   D   G   A   H   G   M   R   S   A   K   G   H   T   F   R   S   S   L   S   V   R  - 336

1009 - CTG CTC AAG TTC TCC CGT GAG AAG AAA GCG GCC AAG ACT CTG GCC ATC GTC GTG GGT GTC TTT GTG CTC TGC  - 1080
 337 -  L   L   K   F   S   R   E   K   K   A   A   K   T   L   A   I   V   V   G   V   F   V   L   C  - 360

1081 - TGG TTC CCT TTC TTC TTT GTC CTG CCG CTC GGC TCC TTG TTC CCG CAG CTG AAG CCA TCG GAG GGC GTC TTC  - 1152
 361 -  W   F   P   F   F   F   V   L   P   L   G   S   L   F   P   Q   L   K   P   S   E   G   V   F  - 384

1153 - AAG GTC ATC TTC TGG CTC GGC TAC TTC AAC AGC TGC GTG AAC CCG CTC ATC TAC CCC TGT TCC AGC CGC GAG  - 1224
 385 -  K   V   I   F   W   L   G   Y   F   N   S   C   V   N   P   L   I   Y   P   C   S   S   R   E  - 408

1225 - TTC AAG CGC GCC TTC CTC CGT CTC CTG CGC TGC CAG TGC CGT CGT CGC CGG CGC CGC CGC CCT CTC TGG CGT  - 1296
 409 -  F   K   R   A   F   L   R   L   L   R   C   Q   C   R   R   R   R   R   R   R   P   L   W   R  - 432

1297 - GTC TAC GGC CAC CAC TGG CGG GCC TCC ACC AGC GGC CTG CGC CAG GAC TGC GCC CCG AGT TCG GGC GAC GCG  - 1368
 433 -  V   Y   G   H   H   W   R   A   S   T   S   G   L   R   Q   D   C   A   P   S   S   G   D   A  - 456

1369 - CCC CCC GGA GCG CCG CTG GCC CTC ACC GCG CTC CCC GAC CCC GAC CCC GAA CCC CCA GGC ACG CCC GAG ATG  - 1440
 457 -  P   P   G   A   P   L   A   L   T   A   L   P   D   P   D   P   E   P   P   G   T   P   E   M  - 480

1441 - CAG GCT CCG GTC GCC AGC CGT CGA AAG CCA CCC AGC GCC TTC CGC GAG TGG AGG CTG CTG GGG CCA TTC CGG  - 1512
 481 -  Q   A   P   V   A   S   R   R   K   P   P   S   A   F   R   E   W   R   L   L   G   P   F   R  - 504

1513 - AGA CCC ACG ACC CAG CTG CGC GCC AAA GTC TCC AGC CTG TCG CAC AAG ATC CGC GCC GGG GGC GCG CAG CGC  - 1584
 505 -  R   P   T   T   Q   L   R   A   K   V   S   S   L   S   H   K   I   R   A   G   G   A   Q   R  - 528

1585 - GCA GAG GCA GCG TGC GCC CAG CGC TCA GAG GTG GAG GCT GTG TCC CTA GGC GTC CCA CAC GAG GTG GCC GAG  - 1656
 529 -  A   E   A   A   C   A   Q   R   S   E   V   E   A   V   S   L   G   V   P   H   E   V   A   E  - 552

1657 - GGC GCC ACC TGC CAG GCC TAC GAA TTG GCC GAC TAC AGC AAC CTA CGG GAG ACC GAT ATT TAA   - 1719
 553 -  G   A   T   C   Q   A   Y   E   L   A   D   Y   S   N   L   R   E   T   D   I  Stp  - 573
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