



CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN α_{2A} ADRENERGIC RECEPTOR

CATALOG NUMBER: HTS096M 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 α_2 adrenergic receptor subfamily members, consisting of α_{2A} , α_{2B} , and α_{2C} , couple primarily to G_i to inhibit cAMP production, and play an important role in regulation of cardiovascular and CNS function. Experiments with α_{2A} -selective agonists and mice lacking α_{2A} demonstrate that activation of α_{2A} results in hypotension, sedation, analgesia, and hypothermia (Kable *et al.*, 2000). Chemicon's α_{2A} 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 at α_{2A} . The membrane preparations exhibit a K_d of 5.4 nM for [3 H]-MK-912. With 5 nM [3 H]-MK-912, 5 μ g/well α_{2A} Membrane Prep typically yields greater than 20-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

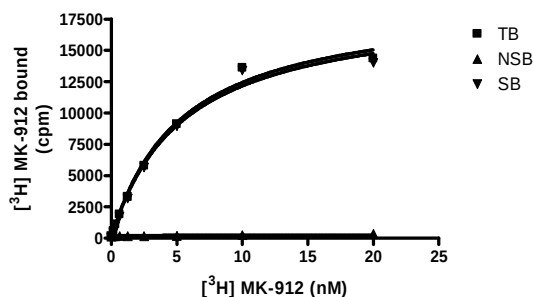


Figure 1. Saturation binding for α_{2A} . 5 μ g/well α_{2A} Membrane Preparation was incubated with increasing amount of 3 H-labeled MK-912 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled rauwolscine. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

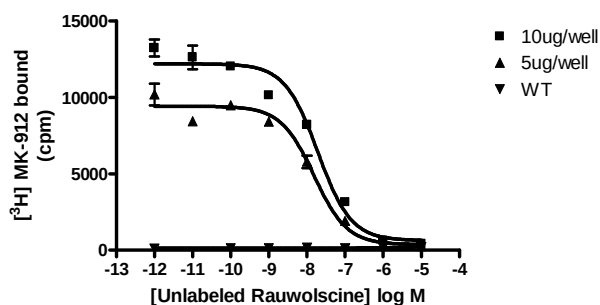


Figure 2. Competition binding for α_{2A} . 5 or 10 $\mu\text{g}/\text{well}$ α_{2A} Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 5 nM ^3H -labeled MK-912 and increasing concentrations of unlabeled rauwolscine. More than 20- fold signal:background was obtained with rauwolscine. Sample data from a representative lot.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with α_{2A} membrane prep and unlabeled rauwolscine.

	10 $\mu\text{g}/\text{well}$	5 $\mu\text{g}/\text{well}$
Signal:background	28.97	34.14
Specific binding (cpm)	11777	9147

SPECIFICATIONS: 1 unit = 5 μg

B_{max} for ^3H MK-912 binding: 71.4 pmol/mg protein

K_d for ^3H MK-912 binding: ~5.4 nM

TRANSFECTION: Full-length human ADRA2A transcript variant 1 cDNA encoding α_{2A} (Accession Number: NM_000681)

HOST CELLS: Chem-1, an adherent mammalian cell line with minimum amount of endogenous α_{2A} 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [³H] MK-912 (Perkin Elmer#: NET-1059)

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

PRESENTATION:

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 20-fold signal:background with ³H-labeled MK-912 at 5 nM

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 1 mg/mL in packaging buffer, and dispensed at 1 mL/vial. Vials were 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. Do not freeze and thaw.

REFERENCES:

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

Kable JW *et al.* (2000) In vivo gene modification elucidates subtype-specific functions of α₂-adrenergic receptors. *J. Pharmacol. Exp. Ther.* **293**: 1-7.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC
PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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