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PRODUCT DATASHEET

ChemiScreen™ α_{2A} Adrenergic Membrane Preparation

CATALOG NUMBER:	HTS096M	**QUANTITY:**	200 units
LOT NUMBER:	21D1412	**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). 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 to α_{2A}.

APPLICATIONS: Radioligand binding assay

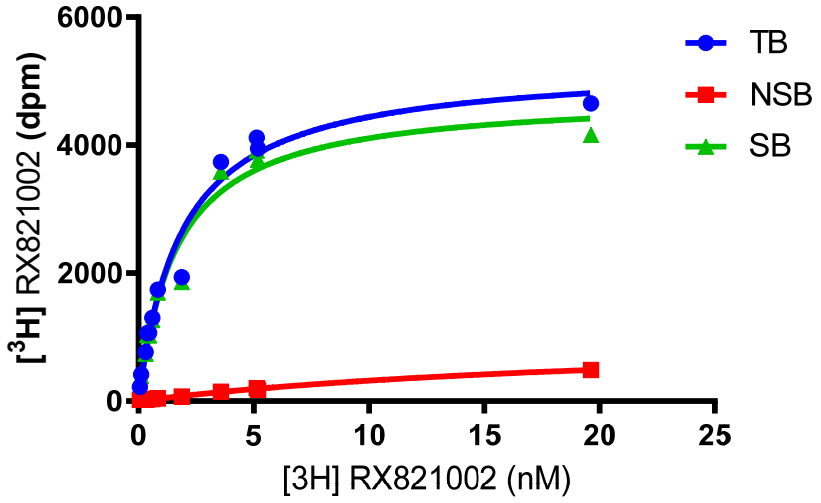

Figure 1. Saturation Binding for α_{2A}. 5 µg/well of α_{2A} Membrane Preparation were incubated with increasing amounts of [^{3}H]-RX821002 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than a 500-fold excess of unlabeled epinephrine. Specific binding (SB) was determined by subtracting NSB from TB. The sample data are from a representative lot.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

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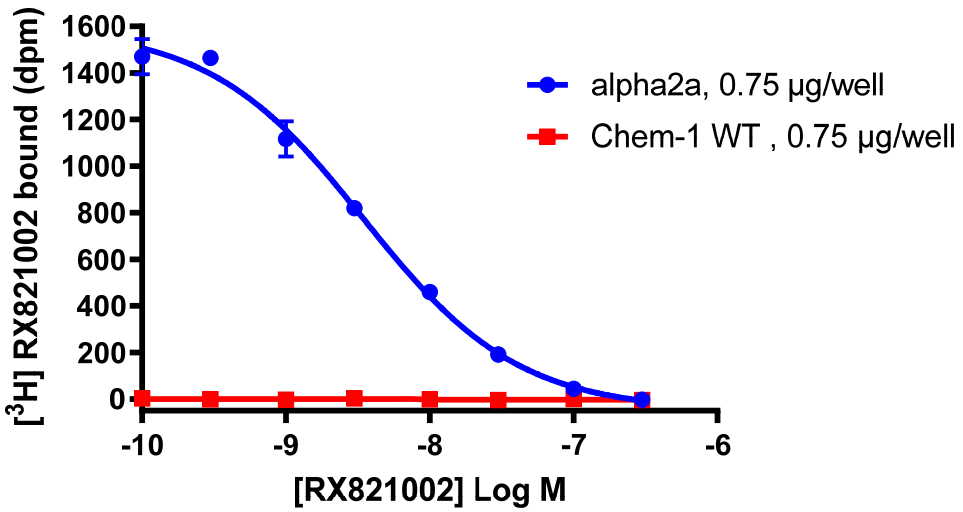

Figure 2. Competition Binding for α_{2A}. 5 or 10 µg/well of α_{2A} Membrane Preparation or Wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 1 nM [^{3}H]-RX821002 and increasing concentrations of unlabeled -RX821002. More than a 20-fold signal:background ratio was obtained. The sample data are from a representative lot.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{3}H]- RX821002 Binding: 82.68 pmol/mg protein
K_d for [^{3}H]- RX821002 Binding: 1.6 nM
Signal:background: >20-fold

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

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable 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 non-binding 96-well plate, and incubated at room temperature for 2 h. Prior to filtration, an FC 96-well harvest plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then dried and counted.

Binding buffer: 50 mM Tris-HCl (pH 7.4), 2 mM MgCl2 and 1 mM EDTA, filtered and stored at 4°C.

Radioligand: [^{3}H]-RX 821002 (PerkinElmer#: NET1153)

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 a 20-fold signal:background ratio with [^{3}H]-MK-912 at 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 1 mg/mL in packaging buffer, dispensed at 1 mL per vial, 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. Kable JW *et al.* (2000). In vivo gene modification elucidates subtype-specific functions of α_2-adrenergic receptors. *J. Pharmacol. Exp. Ther.* 293:1-7.