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PRODUCT DATASHEET

ChemiScreen™ A_1 Adenosine Membrane Preparation

CATALOG NUMBER:	HTS047M	**QUANTITY:**	200 units
LOT NUMBER:	SC20181219	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: Extracellular adenosine mediates a multitude of biological effects, including wakefulness, antiarrythmia, bronchoconstriction and response to ischemia and oxidative stress. A family of four GPCR adenosine receptors, A1, A2A, A2B and A3, is responsible for these effects. The A1 receptor, which couples to Gi/o, is most highly expressed in brain, and mediates endogenous antinociception and neuronal response to hypoxia (Fredholm et al., 2001). A1 is also expressed in kidney, where it contributes to tubuloglomerular feedback. A1 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 A1. The membrane preparations exhibit a Kd of 4.6 nM for [3H]-Cyclopentyl-1, 3-dipropylxanthine, 8-[dipropyl-2, 3-3H (N)]. With 10 µg/well A1 Membrane Prep and 20 nM [3H]-DPCPX, a greater than 6-fold signal-to-background ratio is obtained.

APPLICATIONS: Radioligand binding assay

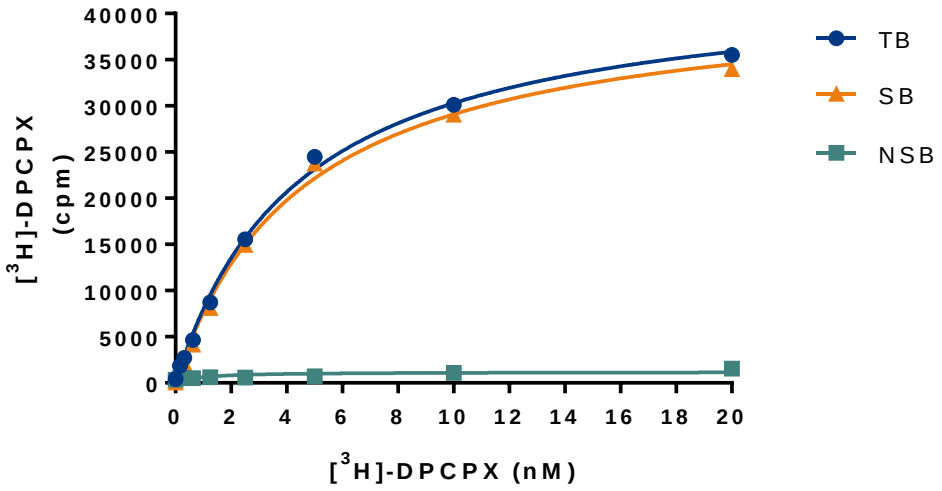

Figure 1. Saturation binding for A_1. 10 µg/well A_1 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled DPCPX in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled DPCPX. Specific binding (SB) was determined by subtracting NSB from TB.

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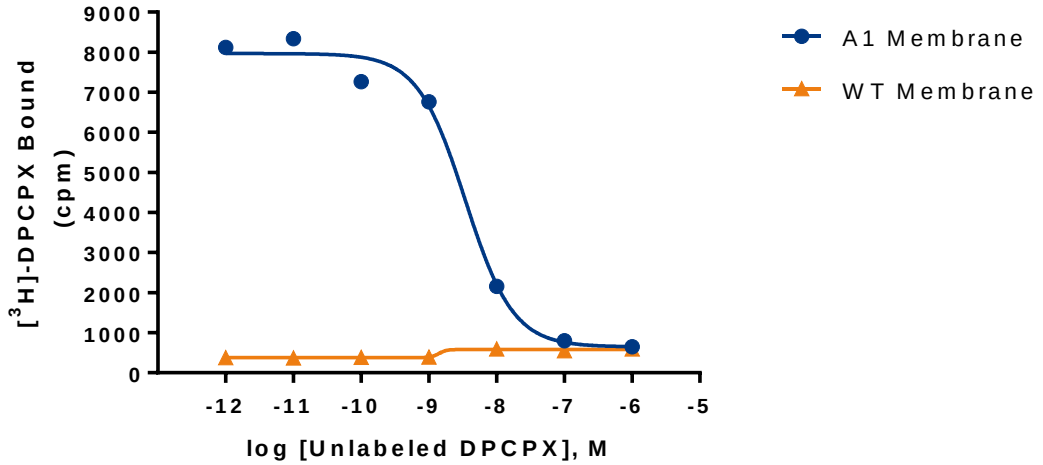

Figure 2. Competition binding for A_1. 10 µg/well A_1 Membrane Preparation (A_1; HTS047M) and wild type Membrane Preparation (WT) were incubated with 1 nM ^{3}H-labeled DPCPX and increasing concentrations of unlabeled DPCPX. More than 6- fold signal:background was obtained with A_1 membranes, whereas no binding was observed with wild-type membranes.

SPECIFICATIONS: 1 unit = 10 µg
B_{max}: 23.28 pmol/mg
K_d: 4.6 nM
Signal:Background: >6-fold

Species: Human A_1 (Accession number S45235)

HOST CELLS: Chem-3, a suspension mammalian cell line without any endogenous A_1 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, and 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] DPCPX (Perkin Elmer# :NET974)

Wash Buffer: 50 mM Tris, pH 7.4, 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 6-fold signal:background with ^{3}H-labeled DPCPX at 1 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in packaging buffer, 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:

1. Fredholm, BB et al. (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53: 527-552.