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PRODUCT DATASHEET

ChemiScreen™ AT_1 Angiotensin II Membrane Preparation

CATALOG NUMBER:	HTS064M	**QUANTITY:**	200 units
LOT NUMBER:	JH1734906	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND: Angiotensin II (Ang II), an octapeptide produced by cleavage of angiotensinogen by angiotensin-converting enzyme, plays a fundamental role in cardiovascular homeostasis. Two GPCRs, AT_1 and AT_2, mediate the effects of AngII, although AT_1 is primarily responsible for the effects of Ang II on renal function and development, thirst, electrolyte and water balance, and arterial blood pressure. Binding of Ang II to AT_1 activates both Gq and Gi (De Gasparo *et al.*, 2000). AT_1 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 antagonists of AT_1 interactions and its ligands.

APPLICATIONS: Radioligand Binding Assay

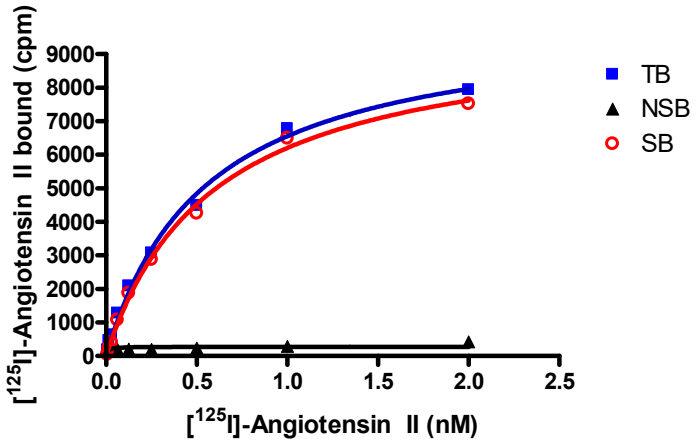

Figure 1. Saturation Binding for AT_1. 5 µg/well AT_1 Membrane Preparation was incubated with increasing amount of ^{125}I labeled Sar^1-Ile^8-Angiotensin II in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Angiotensin II. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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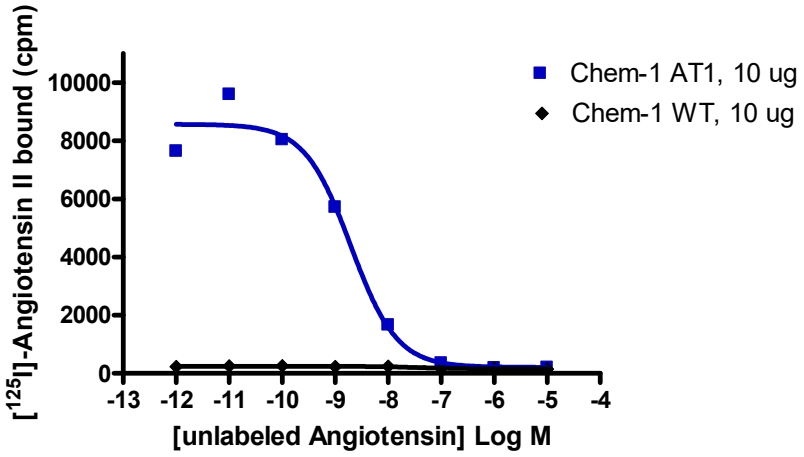

Figure 2. Competition binding for AT_1. AT_1 Membrane Preparation (10 µg/well in a 96-well plate) were incubated with 0.3 nM ^{125}I labeled Sar^1-Ile^8-Angiotensin II and increasing concentrations of unlabeled Angiotensin II, and subjected to filtration binding. Sample data from a representative lot.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I] Angiotensin II binding: 0.45 pmol/mg protein
K_d for [^{125}I] Angiotensin II binding: 0.60 nM

TRANSFECTION: Full-length human AGTR1 cDNA encoding AT_1 (Accession Number: NM_000685).

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous AT_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, a GF/C 96-well filter plate 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°C

Radioligand: [^{125}I]-Sar^1-Ile^8-Angiotensin II. (Perkin Elmer#:NEX-248)

Wash Buffer: 50 mM Hepes, 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 40-fold signal:background with ^{125}I labeled Sar^1-Ile^8-Angiotensin II at 0.3 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein was 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. De Gasparo M *et al*. (2000) International Union of Pharmacology. XXIII. The angiotensin II receptors. *Pharmacol. Rev.* 52: 414-472.