

PRODUCT DATASHEET
ChemiScreen™ AT₂ Angiotensin II Membrane Preparation

CATALOG NUMBER:	HTS208M	QUANTITY:	200 units
LOT NUMBER:	SC692240	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, AT₁ and AT₂, mediate the effects of AngII. Most of the known actions of angiotensin II are mediated through the AT₁ receptor and serve to maintain blood pressure and glomerular filtration rate in the face of extracellular volume depletion (Griendling *et al.* 1996). In vitro and in vivo studies indicated that the AT₂ receptor counterbalances the effect of the AT₁ receptor (Horiuchi *et al.* 1999). Expression of the AT₂ receptor is developmentally regulated: it is highly expressed in various fetal tissues and at a lower density in adult adrenal medulla, brain, and reproductive tissues (Griendling *et al.* 1996). AT₂ expression appears to be re-expressed or up-regulated after vascular injury, myocardial infarction, cardiac failure or wound healing (Matsubara 1998). AT₂ 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 of AT₂. The membrane preparations exhibit a K_d of 0.182 nM for [¹²⁵I]-CGP42112. With 0.25 nM [¹²⁵I]-CGP42112, 5 μg/well AT₂ Membrane Prep typically yields greater than 30-fold signal-to-background ratio.

APPLICATIONS: Radioligand Binding Assay

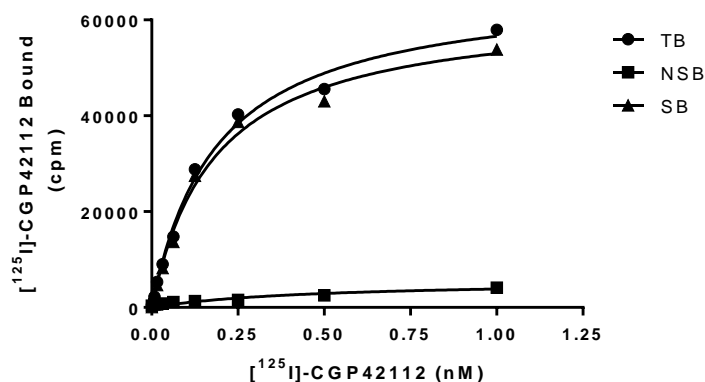


Figure 1. Saturation Binding for AT₂. 5 μg/well AT₂ Membrane Preparation was incubated with increasing amount of [¹²⁵I] CGP42112 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled angiotensin II. Specific binding (SB) was determined by subtracting NSB from TB.

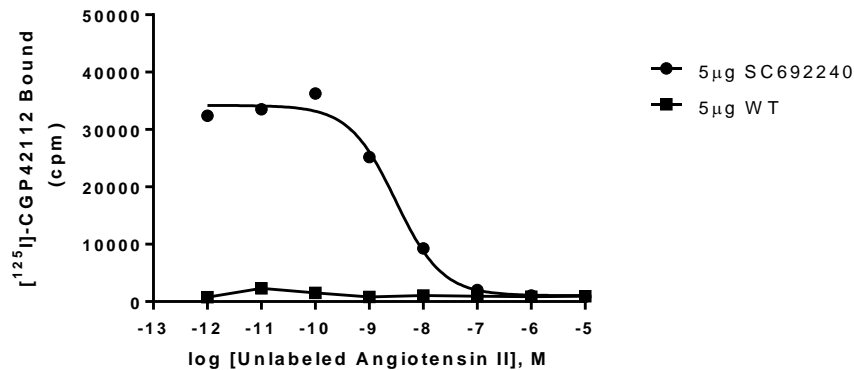


Figure 2. Competition binding for AT₂. 5μg/well AT₂ Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.25 nM [¹²⁵I] CGP42112 and increasing concentrations of unlabeled angiotensin II. More than 30-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 μg
 B_{max} for [¹²⁵I]-CGP42112 binding: 5.13 pmol/mg protein
 K_d for [¹²⁵I]-CGP42112 binding: ~0.182 nM

TRANSFECTION: Full-length human AGTR2 cDNA encoding AT₂ (Accession Number: NM_000686).

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous AT₂ 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 (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM 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: [¹²⁵I]-CGP42112. (PerkinElmer # NEX-324)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM 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 30-fold signal:background with [¹²⁵I] CGP42112 at 0.25 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml 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. Griendling KK *et al.* (1996). Angiotensin receptors and their therapeutic implications. *Annu. Rev. Pharmacol. Toxicol.* 36:281-306.

2. Horiuchi M *et al.* (1999). Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. *Hypertension* 33:613-621.
3. Matsubara H (1998). Pathophysiological role of angiotensin II type 2 receptor in cardiovascular and renal diseases. *Circ. Res.* 83:1182-1191.

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