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PRODUCT DATASHEET

ChemiScreen™ GPR14/Urotensin Ii Receptor Membrane Preparation

CATALOG NUMBER:	HTS033M	**QUANTITY:**	200 units
LOT NUMBER:	1966640	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: Urotensin II, a cyclic 11-13 residue peptide expressed in motoneurons of the spinal cord, acts as a potent vasoconstrictor (Coulouarn et al., 1998; Ames et al., 1999). The effects of urotensin II are mediated by binding to a GPCR, GPR14, which is expressed in endothelium, smooth muscle, heart and pancreas (Ames et al., 1999). Genetic deletion of GPR14 in mice renders aortae refractile to the contractile activity of urotensin II without changing baseline hemodynamics (Behm et al., 2003). Pharmacological inhibition of urotensin II/GPR14 interactions prevents renal insufficiency following renal artery ligation (Clozel et al., 2004). Thus, GPR14 is an attractive target for a number of cardiovascular diseases. EMD Millipore's GPR14 Membrane Preparations are ideal tools for screening for antagonists of urotensin II/GPR14 interactions.

APPLICATIONS: Radioligand binding assay

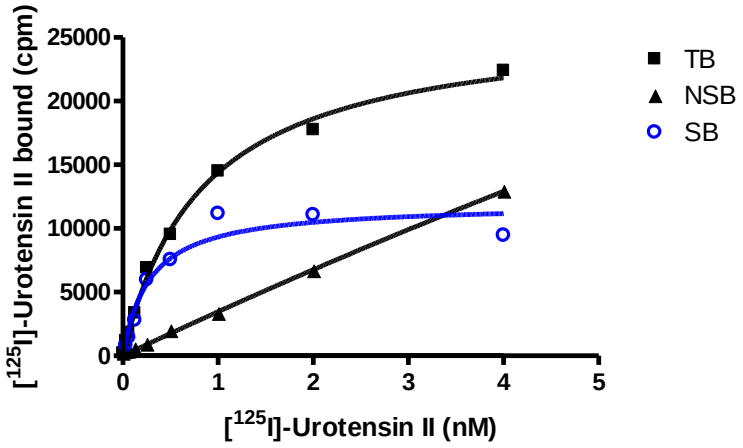

Figure 1. Saturation binding for GPR14. 10 µg/well GPR14 Membrane Preparation was incubated with 2-fold dilutions of ^{125}I-labeled urotensin II in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled urotensin II. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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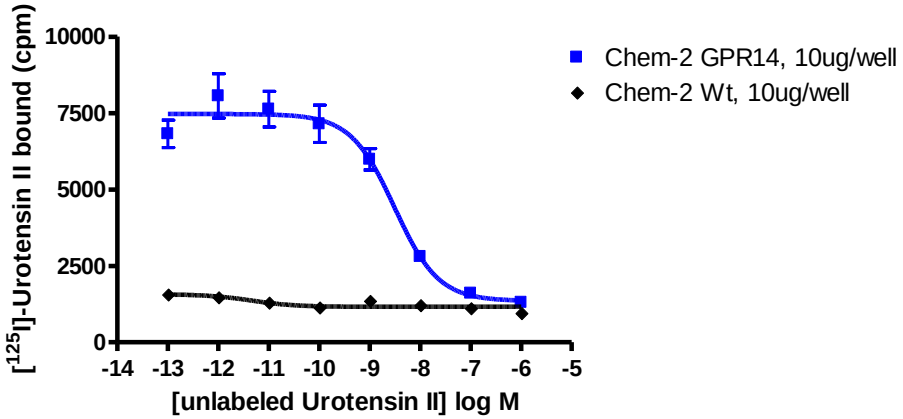

Figure 2. Competition binding for GPR14. 10 µg/well GPR14 Membrane Preparation was incubated with 0.3 nM ^{125}I-labeled urotensin II and increasing concentrations of unlabeled urotensin II, and more than 5-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg
Bmax for [125I] urotensin II binding: 0.5 pmol/mg protein
Kd for [125I] urotensin II binding: 0.28 nM
Signal:background: >5-fold

TRANSFECTION: Full-length human GPR14 cDNA (Accession Number: AF140631)

Species: Human

HOST CELLS: Chem-2, a suspension mammalian cell line with no endogenous GPR14 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 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]-urotensin II (Perkin Elmer # NEX379)

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 5-fold signal:background with 125I-labeled urotensin II at 0.3 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 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. Ames R.S., et al. (1999) Human urotensin-II is a potent vasoconstrictor and agonist for the orphan receptor GPR14. Nature 401:282-286.
2. Behm D.J., et al. (2003) Deletion of the UT receptor gene results in the selective loss of urotensin-II contractile activity in aortae isolated from UT receptor knockout mice. Br. J. Pharmacol. 139: 464-72.
3. Clozel M., et al. (2004) Pharmacology of the urotensin-II receptor antagonist palosuran (ACT- 058362; 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulfate salt): first demonstration of a pathophysiological role of the urotensin system. J. Pharmacol. Exp. Ther. 311: 204-12.
4. Coulouarn Y, et al. (1998) Cloning of the cDNA encoding the urotensin II precursor in frog and human reveals intense expression of the urotensin II gene in motoneurons of the spinal cord. Proc. Natl. Acad. Sci. USA 95: 15803-8.

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