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PRODUCT DATASHEET

ChemiScreen™ ET_B Endothelin Membrane Preparation

CATALOG NUMBER:	HTS046M	**QUANTITY:**	200 units
LOT NUMBER:	2071128	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND:

The endothelin family of peptides are potent vasoconstrictors synthesized by endothelial cells in both constitutive and inducible pathways. The biological actions of endothelins are mediated by two GPCRs, ET_A and ET_B (Davenport, 2002). Whereas ET_A is the predominant receptor on smooth muscle cells and thus is the primary mediator of the vasoconstrictor activity, ET_B is found on endothelial cells and mediates vasodilation (Nilsson *et al.*, 1997). In addition, ET_B has also been linked to renal failure, congestive heart failure, atherosclerosis, and pulmonary hypertension (D'Orléans-Juste, *et al.,* 2002). A mutation in ET_B has been found to cause Hirschsprung disease-2, a degenerative disease of the digestive tract (Puffenberger *et al*, 1994). The cloned human ET_B-expressing cell line is made in the CHO host, which supports high levels of recombinant ET_B expression on the cell surface. Thus, the ET_B Membrane Preparation is an ideal tool for screening for compounds binding to ET_B. The membrane preparations exhibit a Kd of 0.03-0.06 nM for [^{125}I]-endothelin-1. With 0.2 nM [^{125}I]-endothelin-1, 10 µg/well ET_B Membrane Prep yields greater than 5 fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

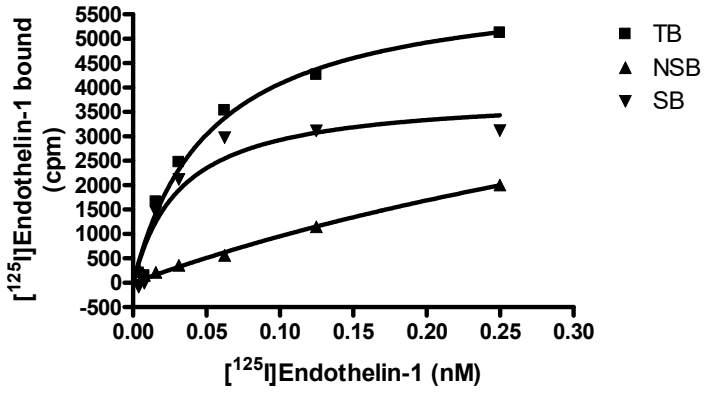

Figure 1. Saturation Binding for ET_B. 10 µg/well ET_B Membrane Preparation was incubated with increasing amount of [^{125}I]Endothelin-1 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Endothelin-1. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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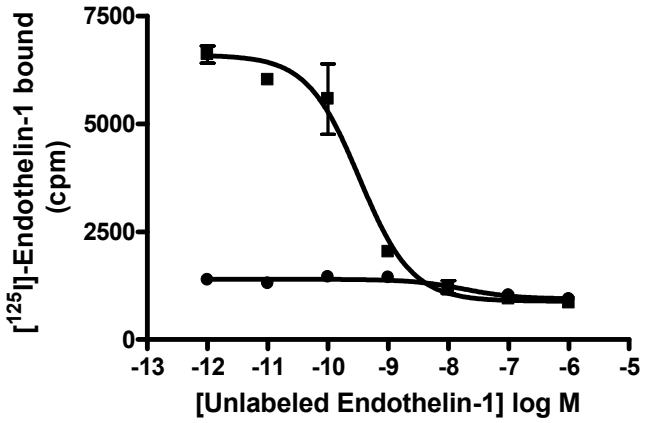

Figure 2. Competition binding for ET_B. ETB Membrane Preparation (10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.2 nM [^{125}I]-Endothelin-1 and increasing concentrations of unlabeled Endothelin-1, and more than 5- fold signal:background was obtained. Representative sample data.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of ET_A membrane prep.

SPECIFICATIONS: 1 unit = 10 µg
B_{max}: 0.49 pmol/mg
K_d: 0.03-0.06 nM

Species: Human ET_B isoform 1 (Accession Number: NM_00015)

HOST CELLS: CHO-K1 (Chinese hamster ovary cells)

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 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]Endothelin-1 (Perkin Elmer # NEX259)

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 an unit is the amount of membrane that will yield greater than 5-fold signal:background with ^{125}I-labeled Endothelin-1 at 0.25 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 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. Davenport AP (2002) International Union of Pharmacology. XXIX. Update on Endothelin Receptor Nomenclature. *Pharmacol. Rev.* 54: 219-226.
2. Nilsson T, *et al.* (1997) Presence of contractile endothelin-A and dilatory endothelin-B receptors in human cerebral arteries. *Neurosurgery.* 40: 346–351;comment 351–353
3. D'Orléans-Juste P, *et al* (2002) Function of the endothelin$_B$ receptor in cardiovascular physiology and pathophysiology. *Pharmacol Ther.* 95: 221-238.
4. Puffenberger EG, *et al* (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* 79: 1257-1266.