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PRODUCT DATASHEET

ChemiScreen™ ET_A Endothelin Membrane Preparation

CATALOG NUMBER:	HTS070M	**QUANTITY:**	200 units
LOT NUMBER:	22K0502	**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. ET_A is the predominant receptor on smooth muscle cells and thus is the primary mediator of the vasoconstrictor activity of endothelins in vivo. Endothelin receptors are also found in the epithelium and central nervous system (Davenport, 2002). ET_A 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 ET_A interactions with its ligands.

APPLICATIONS: Radioligand binding assay

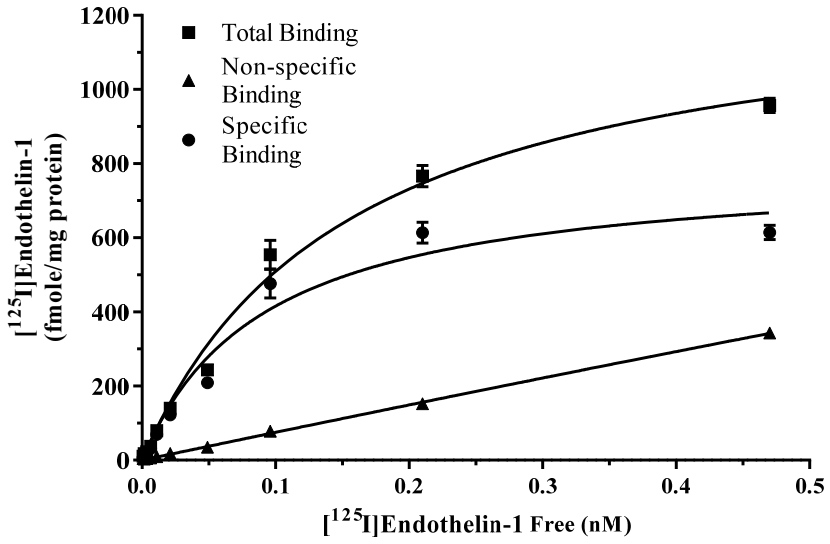

Figure 1. Saturation Binding for ET_A. 5 µg/well ET_A Membrane Preparation was incubated with increasing amount of ^{125}I-labeled Endothelin-1 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Endothelin-1. Specific binding (SB) was determined by subtracting NSB from TB.

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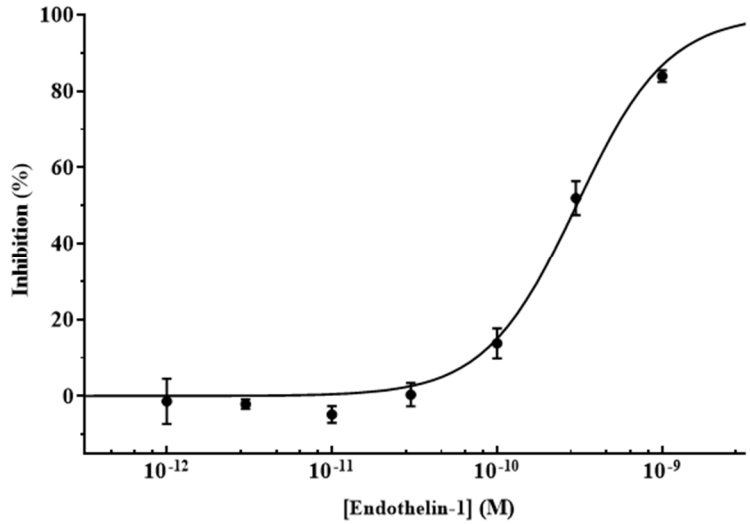

Figure 2. Competition binding for ET_A. 10 µg/well ET_A Membrane Preparation (HTS070M) was incubated with 0.03 nM ^{125}I-labeled Endothelin-1 and increasing concentrations of unlabeled Endothelin-1. A 3-fold signal:background ratio was obtained.

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}: 0.8 pmol/mg protein
K_d: 92 pM
Signal:background: 3-fold

Species: Full-length human EDNRA cDNA encoding ET_A (Accession Number: S63938).

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable endogenous endothelin receptor 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 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 NEX-259)

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 a 3-fold signal:background with ^{125}I-labeled Endothelin-1 at 0.03 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 -80ºC.

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.

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