

PRODUCT DATASHEET
ChemiScreen™ SST₃ Receptor Membrane Preparation

CATALOG NUMBER:	HTS171M	QUANTITY:	200 units
LOT NUMBER:	2278645	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: Somatostatin is a 14 or 28 amino acid regulatory peptide that inhibits hormone secretion from the pituitary, pancreas, and other endocrine sites. A family of 6 GPCRs, sst₁, sst_{2A}, sst_{2B}, sst₃, sst₄ and sst₅, mediate the biological activity of somatostatins. The somatostatin receptors couple to G_i to inhibit cAMP production, and also increase MAP kinase signalling. Several tumors have been shown to overexpress somatostatin receptors, and binding of somatostatin to these tumor cells stimulates or inhibits proliferation, depending on the receptor subtypes expressed (Olias *et al.*, 2004). However, sst₃ appears to promote apoptosis, and expression of sst₃ was found to be lower in gastric cancer cells than in normal gastric mucosa, in proportion to susceptibility to apoptosis induced by somatostatin analogs (Sharma *et al.*, 1996; Hu *et al.*, 2004). In addition, sst₃ is expressed in smooth muscle cells and mediates gastrointestinal contractions. sst₃ 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 sst₃ interactions and its ligands.

APPLICATIONS: Radioligand Binding Assay

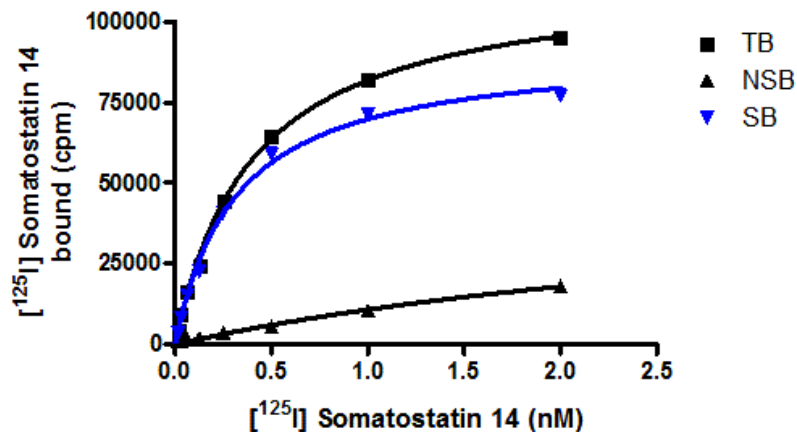


Figure 1. Saturation Binding for SST₃. 5.0 µg/well sst₃ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-somatostatin 14 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled somatostatin. Specific binding (SB) was determined by subtracting NSB from TB. Data from a representative Lot.

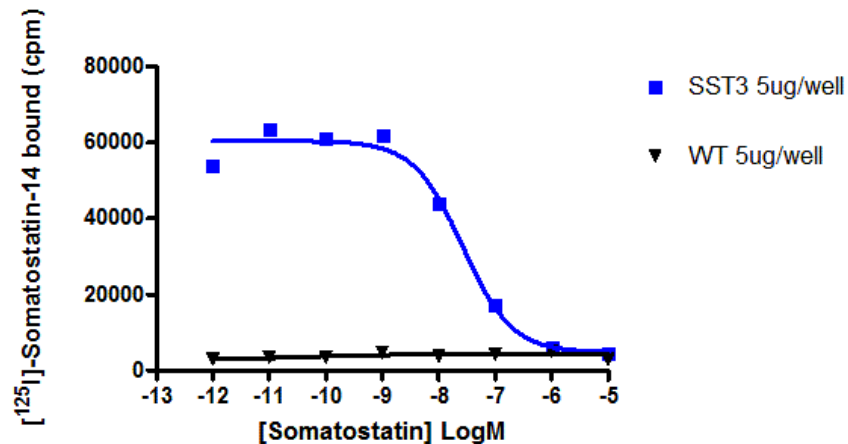


Figure 2. Competition Binding for SST₃. 5µg/well sst₃ Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.5 nM ¹²⁵I-labeled Somatostatin 14 and increasing concentrations of unlabeled somatostatin. More than 12-fold signal:background was obtained. Data from a representative Lot.

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [¹²⁵I]-Somatostatin Binding: 7.5 pmol/mg protein
 K_d for [¹²⁵I]-Somatostatin Binding: 0.3 nM
 Signal:background: ≥12-fold

TRANSFECTION: Full-length Human SSTR3 cDNA encoding SST₃ (Accession Number: NM_001051)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous SST₃ 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 (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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [¹²⁵I]-Somatostatin 14. (Perkin Elmer # NEX-389)

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 12-fold signal:background with ¹²⁵I labeled somatostatin 14.

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 1 mg/mL packaging buffer, dispensed at 1 mL per vial, 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Hu C *et al.* (2004) The effect of somatostatin and SSTR3 on proliferation and apoptosis of gastric cancer cells. *Cancer Biol. Ther.* 3(8): 726-730.
2. Olias G *et al.* (2004) Regulation and function of somatostatin receptors. *J. Neurochem.* 89: 1057-1091.
1. Sharma K *et al.* (1996) Subtype-selective induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. *Mol. Endocrinol.* 13: 82-90.

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