

PRODUCT DATASHEET
ChemiScreen™ SST₁ Somatostatin Membrane Preparation

CATALOG NUMBER:	HTS170M	QUANTITY:	200 units
LOT NUMBER:	2202689	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: Somatostatin, a 14 or 28 amino acid peptide, inhibits hormone secretion from exocrine glands, and displays an antiproliferative activity on a number of cell types. A family of 5 G_i-coupled receptors mediates the effects of somatostatin. The classical activity of somatostatin of inhibiting growth hormone release from the pituitary appears to be mediated by sst₁. In addition, sst₁ functions as an inhibitory autoreceptor on somatostatin neurons in the hippocampus, hypothalamus, basal ganglia and retina (Thermos *et al.*, 2006). The somatostatin receptors are targets for anticancer drugs and for treatment of hormonal dysfunction. sst₁ 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 sst₁. The membrane preparations exhibit a K_d of 1.4 nM for [¹²⁵I]-somatostatin-14. With 0.5 nM [¹²⁵I]-somatostatin-14, 5 μg/well sst₁ Membrane Prep yields greater than 12 fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

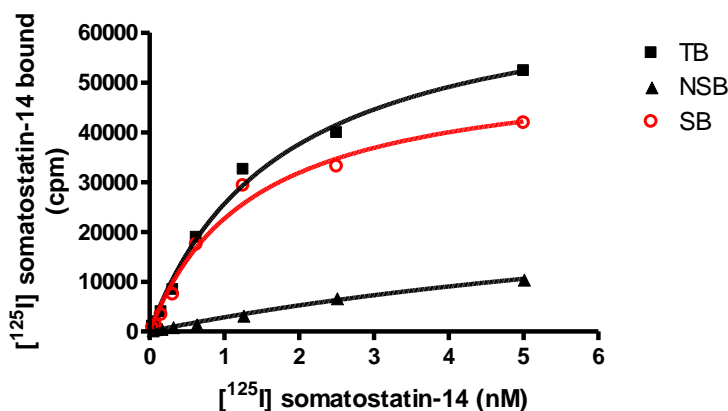


Figure 1. Saturation binding for sst₁. 5 ug/well sst₁ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-somatostatin-14 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled somatostatin-14. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative Lot.

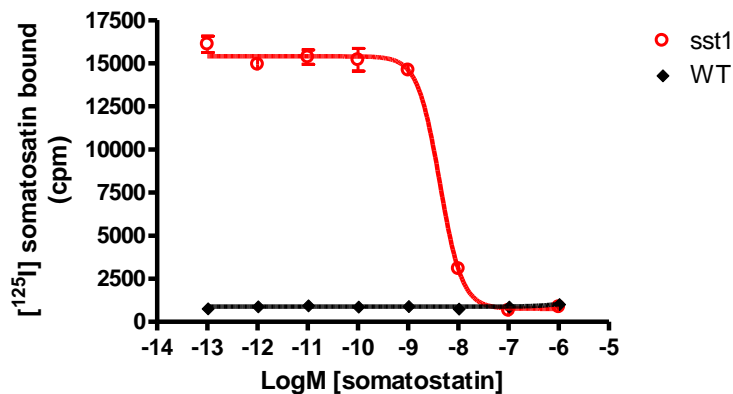


Figure 2. Competition binding for sst₁. SST₁ Membrane Preparation (5 µg/well) and Wild-Type Chem-1 membrane preparation (10 µg/well) were incubated with 0.5 nM [¹²⁵I]-somatostatin-14 and increasing concentrations of unlabeled somatostatin, and more than 12-fold signal:background was obtained. Sample data from a representative Lot.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of sst₁ membrane prep.

	5 µg/well
Signal:background	20.4
Specific binding (cpm)	14,647

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
 B_{max} 8.4 pmol/mg
 K_d 1.4 nM

Species: Human sst₁ (Accession number NM_000868)

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

Radioligand: [¹²⁵I]- somatostatin-14 (PerkinElmer NEX389)

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 12-fold signal:background with [¹²⁵I]-somatostatin-14 at 0.5 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane protein was 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. Theros K *et al.* (2006) The somatostatin sst₁ receptor: an autoreceptor for somatostatin in brain and retina? *Pharmacol. Ther.* 110: 455-464.

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