

PRODUCT DATASHEET
ChemiScreen™ SST₅ Somatostatin Membrane Preparation

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|------------------------|---------|------------------------------|---------------|
| CATALOG NUMBER: | HTS139M | QUANTITY: | 200 units |
| LOT NUMBER: | 22H0106 | VOLUME/CONCENTRATION: | 1 mL, 1 mg/mL |

BACKGROUND: Somatostatin (sst) is a multifunctional peptide with two biologically active forms, sst-14 and sst-28, which are synthesized in neurons throughout the brain as well as in peripheral tissues such as the pancreas and the gut (Gillies, 1997). SST exerts a diverse array of effects that include inhibition of endocrine secretion, modulation of neurotransmission, and regulation of cell proliferation by stimulating a family of five G-protein-coupled receptors. Somatostatin receptor sst₅ is an inhibitory G protein-coupled receptor that exerts a strong cytostatic effect on various cell types. In mice, sst₅ mediates inhibition by somatostatin of pancreatic insulin secretion and contributes to the regulation of glucose homeostasis and insulin sensitivity (Strowski *et al.* 2003). In addition, deficiency of sst₅ leads to subtype-selective sexually dimorphic changes in the expression of both brain and pancreatic somatostatins (Ramirez *et al.* 2004). sst₅ 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 SST₅.

APPLICATIONS: Radioligand Binding Assay

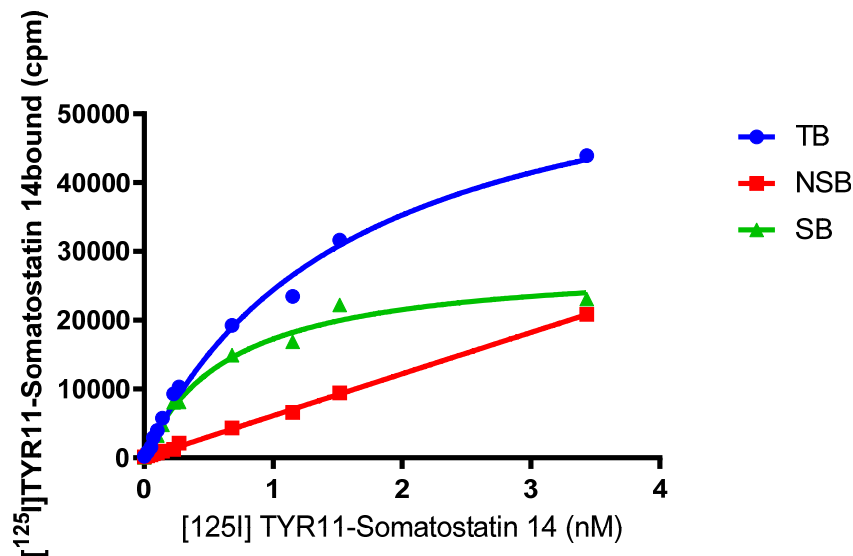


Figure 1. Saturation Binding for SST₅. 5 µg/well of SST₅ Membrane Preparation were incubated with increasing amounts of [¹²⁵I]-Somatostatin 14 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess of unlabeled somatostatin. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

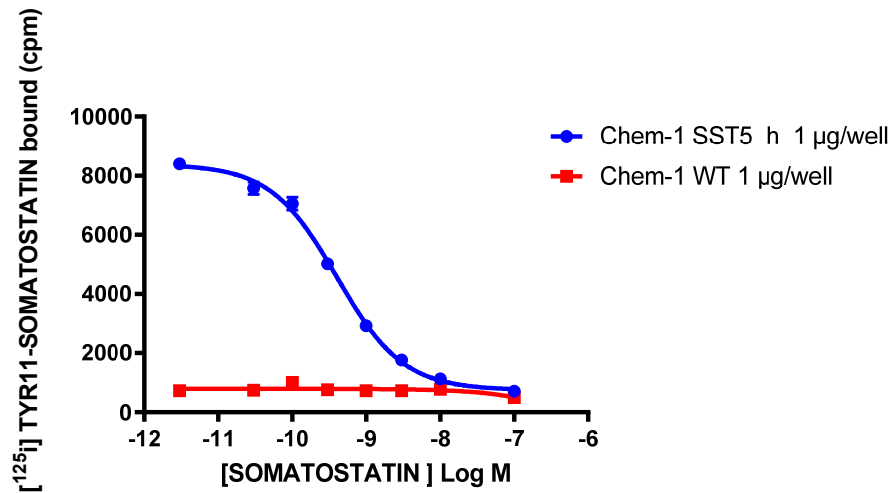


Figure 2. Competition binding for SST5. 1 µg/well of SST5 Membrane Preparation or Wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.5 nM [¹²⁵I]-Somatostatin 14 and increasing concentrations of unlabeled somatostatin. A 10-fold signal:background ratio was obtained. Sample data from a representative lot.

SPECIFICATIONS: 1 unit = 5 µg

B_{max} for [¹²⁵I]-Somatostatin Binding: 8.3 pmol/mg protein

K_d for [¹²⁵I]-Somatostatin Binding: ~0.65 nM

TRANSFECTION: Full-length human SSTR5 cDNA encoding sst5 (Accession Number: NM_001053.1)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous sst5 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 non-binding 96-well plate, and incubated at room temperature for 2 h. Prior to filtration, an FC 96-well harvest plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then 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#: 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 a 10-fold signal:background ratio 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: Membranes proteins were adjusted to 1 mg/mL in 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. Gillies G (1997). Somatostatin: the neuroendocrine story. *Trends Pharmacol. Sci.* 18(3):87-95.
2. Strowski MZ *et al.* (2003). Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. *Mol. Endocrinol.* 17:93–106.
3. Ramirez, JL *et al.* (2004). Deficiency of somatostatin (SST) receptor type 5 (SSTR5) is associated with sexually dimorphic changes in the expression of SST and SST receptors in brain and pancreas. *Mol. Cell. Endocrinol.* 221:105–119.

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