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PRODUCT DATASHEET

ChemiScreen™ SST_2 Receptor Membrane Preparation

CATALOG NUMBER:	HTS028M	**QUANTITY:**	200 units
LOT NUMBER:	SC060773	**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 sst_2 mRNA is predominantly expressed in central nervous system. A study using sst_2 knock-out mice has found increased anxiety-related behavior, while locomotor and exploratory activity was decreased in stress-inducing situations (coupled with an increase in pituitary ACTH release, a regulator of the stress response) (Viollet *et al.*, 2000). In the periphery, inhibition of glucagon release by SST in mouse islets is primarily mediated via SST_2 (Strowski *et al.*, 2000). In addition, endogenous SST functions through SST_2 to suppress gastric acid secretion through inhibition of gastrin activity (Martinez *et al.*, 1998). SST_2 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 SST_2. The membrane preparations exhibit a Kd of 1.5 nM for [^{125}I]-Somatostatin. With 0.75 nM [^{125}I]-Somatostatin, 5 µg/well of SST_2 Membrane Prep typically yields greater than a 14-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

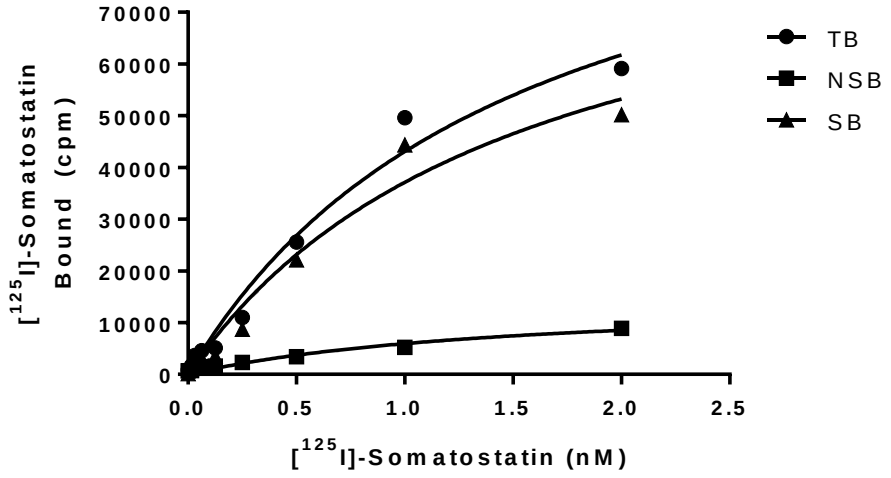

Figure 1. Saturation Binding for SST_2. 5 µg/well of SST_2 Membrane Preparation was incubated with increasing amounts of [^{125}I]-Somatostatin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled somatostatin 14. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC060773.

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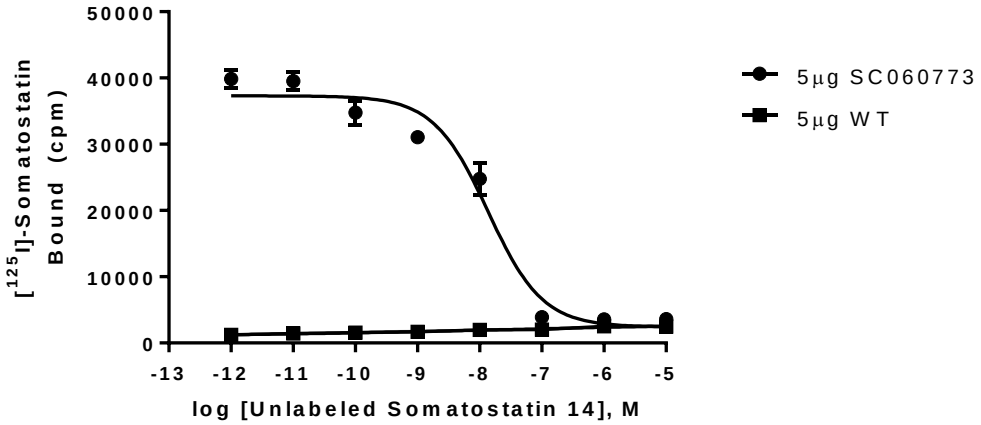

Figure 2. Competition Binding for SST_2. 5 µg/well of SST_2 Membrane Preparation ore Wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.75 nM [^{125}I]-Somatostatin and increasing concentrations of unlabeled somatostatin 14. More than a 14-fold signal:background ratio was obtained. The data are from a representative sample of lot SC060773.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I]-Somatostatin Binding: 7.7 pmol/mg protein
K_d for [^{125}I]-Somatostatin Binding: 1.5 nM
Signal:background: ≥14-fold

TRANSFECTION: Full-length human sst_2 cDNA encoding sst_2 (Accession Number: NM_001050.2)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous SST_2 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes were 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 for 2 h at room temperature. Prior to filtration, an FC 96-well harvest plate was coated with 0.33% polyethylenimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions were transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted to determine the amount of receptor-associated radioligand binding.

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]-Somatostatin (PerkinElmer#: NEX389)

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 a 14-fold signal:background ratio with [^{125}I]-Somatostatin at 0.75 nM.

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. Gillies G (1997). Somatostatin: the neuroendocrine story. *Trends Pharmacol. Sci.* 18:87-95.

2. Martinez V *et al.* (1998). High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. *Gastroenterology* 114:1125-1132.

3. Strowski MZ *et al.* (2000). Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology* 141:111-117.

4. Viollet C *et al.* (2000). Involvement of sst_2 somatostatin receptor in locomotor, exploratory activity and emotional reactivity in mice *Eur. J. Neurosci*. 12:3761-3770.

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