

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN RECOMBINANT sst5 SOMATOSTATIN RECEPTOR

CATALOG NUMBER:	HTS139M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION PER VIAL:	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). Millipore's 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. The membrane preparations exhibit a Kd of 1.2 nM for [¹²⁵I]-Somatostatin 14. With 0.5 nM [¹²⁵I]-Somatostatin 14, 5 μg/well sst5 Membrane Prep typically yields greater than 10-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

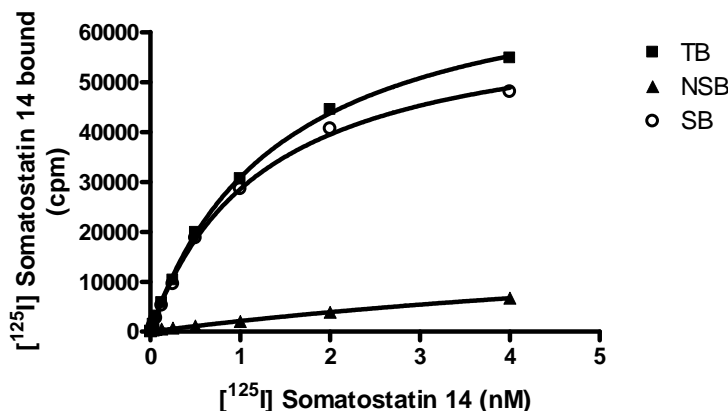


Figure 1. Saturation binding for sst5. 5 μg/well sst5 Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled Somatostatin 14 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled somatostatin. Specific binding (SB) was determined by subtracting NSB from TB.

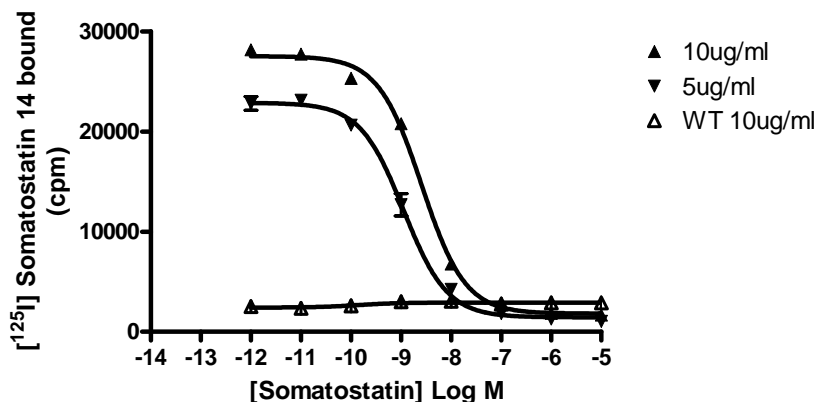


Figure 2. Competition binding for sst5. 10 and 5µg/well sst5 Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.5 nM ¹²⁵I-labeled Somatostatin 14 and increasing concentrations of unlabeled somatostatin. More than 10-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of sst5 Receptor membrane prep.

	10 µg/well	5 µg/well
Signal:background	15.2	15.7
Specific binding (cpm)	25710	21425

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [¹²⁵I]-Somatostatin binding: 10.4 pmol/mg protein
 K_d for [¹²⁵I]-Somatostatin binding: ~1.2 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 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 10-fold signal:background with ¹²⁵I labeled somatostatin 14 at 0.5 nM

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein were adjusted to 1 mg/ml in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES: Gillies G (1997) Somatostatin: the neuroendocrine story. *Trends Pharmacol. Sci.* 18(3):87-95.

Strowski MZ *et al.* (2003). Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. *Mol. Endocrinol.* 17: 93–106.

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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