

**CHEMISCREEN[™] MEMBRANE PREPARATION
RECOMBINANT HUMAN SST₄ SOMATOSTATIN RECEPTOR**

CATALOG NUMBER:	HTS125M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION:	2 mL, 0.5 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). Somatostatin has been implicated in seizure susceptibility in animal models, and activation of sst₄ with selective agonists increases seizure activity (Moneta *et al.*, 2002). Chemicon's SST₄ 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 SST₄ interactions with somatostatin. The membrane preparations exhibit a K_d of 1.78 nM for [¹²⁵I]-Somatostatin 14. With 5 μg/well SST₄ Membrane Prep and 1 nM [¹²⁵I]-Somatostatin 14, a greater than 20-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay, and GTPγS binding.

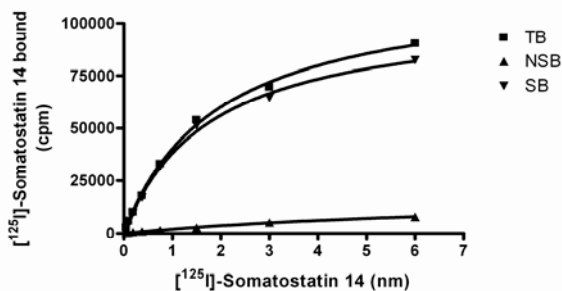


Figure 1. Saturation binding for SST₄. 5 μg/well SST₄ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-Somatostatin 14 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of more than 5000-fold excess unlabeled somatostatin. Specific binding (SB) was determined by subtracting NSB from TB.

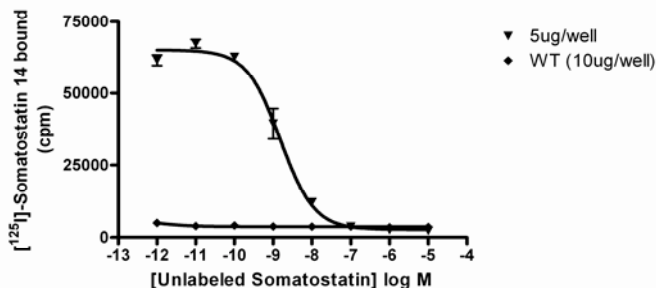


Figure 2. Competition binding for SST₄. SST₄ Membrane Preparation (5 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 1 nM [¹²⁵I]-Somatostatin 14 and increasing concentrations of unlabeled somatostatin, and more than 20-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of SST₄ membrane prep.

	5 µg/well
Signal:background	24.0
Specific binding (cpm)	62319

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
Bmax: 28.2 pmol/mg
K_d: 1.78 nM

Species: Human SSTR4 encoding SST₄ (Accession Number: NM_001052)
HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous SST₄ 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, a GF/C 96-well filter plate 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 # 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 an unit is the amount of membrane that will yield greater than 20-fold signal:background with ¹²⁵I-labeled somatostatin 14 at 1 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membranes protein were adjusted to the indicated concentration in 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: Moneta D *et al.* (2002) Somatostatin receptor subtypes 2 and 4 affect seizure susceptibility and hippocampal excitatory neurotransmission in mice. *Eur. J. Neurosci.* 16: 843-9.

Olias G *et al.* (2004) Regulation and function of somatostatin receptors. *J. Neurochem.* 89: 1057-1091.

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