



CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN SST₃ SOMATOSTATIN RECEPTOR

CATALOG NUMBER: HTS171M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 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). However, sst₃ appears to promote apoptosis, and expression of sst₃ was found to be lower in gastric cancer cells than in normal gastric mucosa, in proportion to susceptibility to apoptosis induced by somatostatin analogs (Sharma *et al.*, 1996; Hu *et al.*, 2004). In addition, sst₃ is expressed in smooth muscle cells and mediates gastrointestinal contractions. Millipore's sst₃ 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 sst₃ interactions and its ligands. The membrane preparations exhibit a K_d of 0.7 nM for [¹²⁵I]-somatostatin 14. With 0.5 nM [¹²⁵I]-Somatostatin 14, 5 µg/well sst₃ Membrane Prep typically yields greater than 20-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

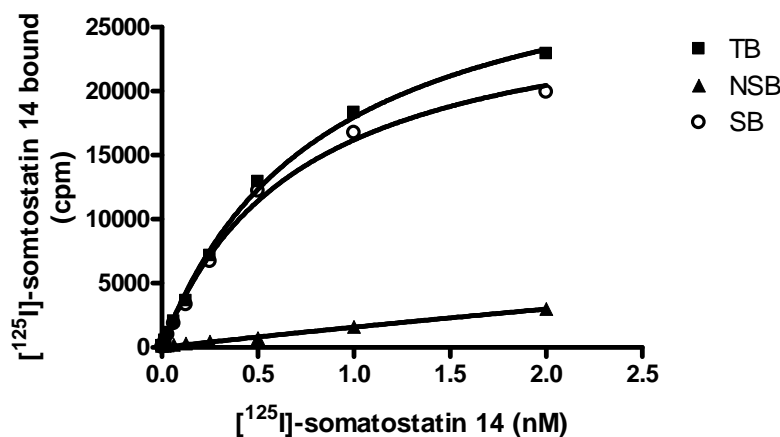


Figure 1. Saturation binding for sst₃. 5.0 µg/well sst₃ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-somatostatin 14 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled somatostatin. Specific binding (SB) was determined by subtracting NSB from TB. Data from a representative Lot.

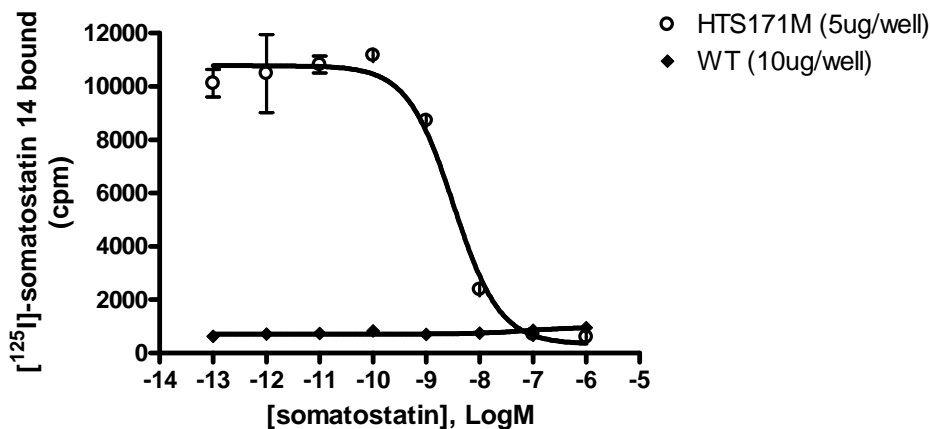


Figure 2. Competition binding for sst₃. 5µg/well sst₃ Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.5 nM ¹²⁵I-labeled Somatostatin 14 and increasing concentrations of unlabeled somatostatin. More than 20-fold signal:background was obtained. Data from a representative Lot.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with 5 µg/well membrane prep.

	5 µg/well
Signal:background	32
Specific binding (cpm)	10447

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
Bmax: 5.1 pmol/mg
K_d: 0.7 nM

Species: Human SSTR3 cDNA encoding SST₃ (Accession Number: NM_001051)

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 (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

PRESENTATION:

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 20-fold signal:background with ¹²⁵I labeled somatostatin 14.

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:

Hu C *et al.* (2004) The effect of somatostatin and SSTR3 on proliferation and apoptosis of gastric cancer cells. *Cancer Biol. Ther.* 3(8): 726-730.

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

Sharma K *et al.* (1996) Subtype-selective induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. *Mol. Endocrinol.* 13: 82-90.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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