

PRODUCT DATASHEET

ChemiScreen™ Y₄ Neuropeptide Y Receptor Membrane Preparation

CATALOG NUMBER:	HTS204M	QUANTITY:	200 units
LOT NUMBER:	2262821	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: The NPY family consists of three 36-amino acid peptides, neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP), which bind to the NPY receptor family of G protein-coupled receptors. Five NPY receptors, Y₁, Y₂, Y₄, Y₅ and Y₆, have been defined at the molecular level, and each signals primarily through Gi/o. Binding of NPY family peptides to NPY receptors mediates a variety of physiological effects, including promotion of food intake, decreased anxiety, inhibition of neurotransmitter and hormone release, vasoconstriction, and gut motility. Y₄ binds preferentially to PP, with significant binding to NPY and PYY (Michel *et al.*, 1998). Y₄ plays a role in control of reproduction, as Y₄-null, ob/ob double knockout mice have increased fertility relative to ob/ob mice with functional Y₄ (Sainsbury *et al.*, 2002). In addition, mice lacking Y₄ display resistance to weight gain induced by high fat diet, and mice lacking both Y₂ and Y₄ are resistant to the enhanced weight gain exhibited by Y₁-null mice fed a high fat diet (Sainsbury *et al.*, 2006). The Y₄ 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 Y₄. The membrane preparations exhibit a K_d of 0.35 nM for [¹²⁵I]-Pancreatic Polypeptide. With 0.4 nM [¹²⁵I]-Pancreatic Polypeptide, 5 μg/well Y₄ Membrane Prep typically yields greater than 20-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay.

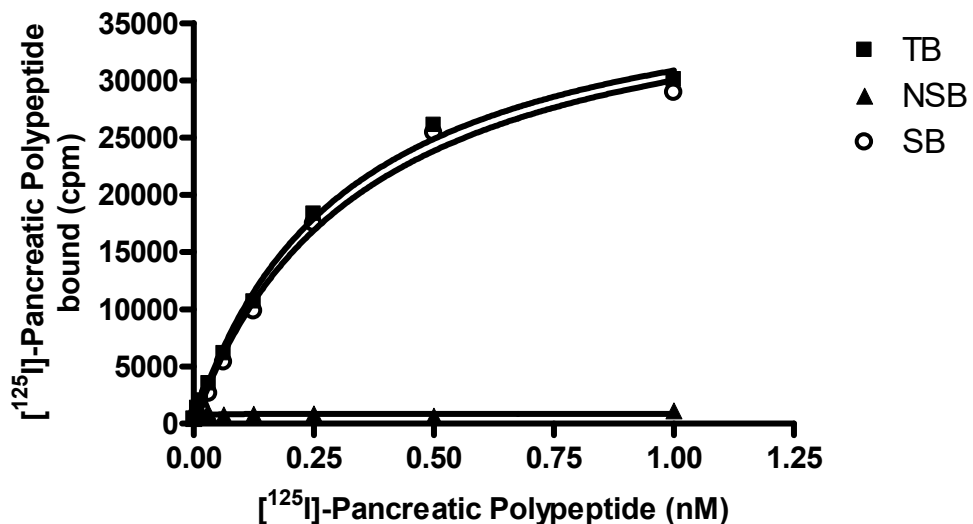


Figure 1. Saturation binding for Y₄. 5 μg/well Y₄ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-labeled Pancreatic Polypeptide in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Pancreatic Polypeptide. Specific binding (SB) was determined by subtracting NSB from TB.

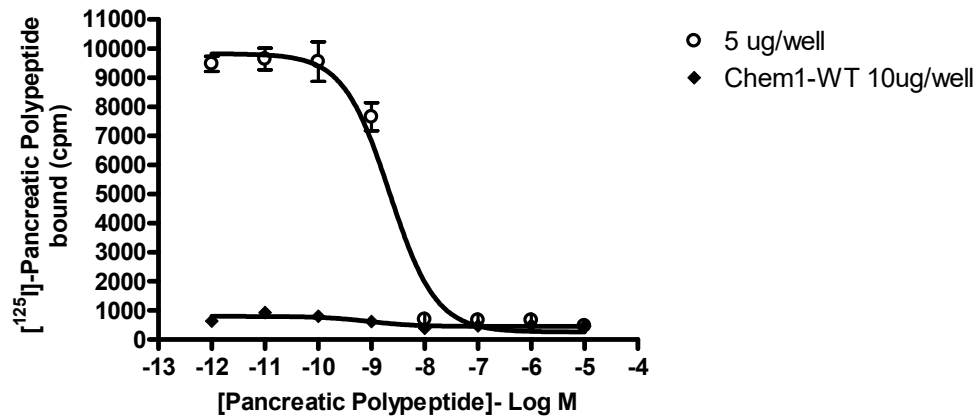


Figure 2. Competition binding for Y₄. 5µg/well Y₄ Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.4 nM [¹²⁵I]-labeled Pancreatic Polypeptide and increasing concentrations of unlabeled Pancreatic Polypeptide. More than 20-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 µg

B_{max} for [¹²⁵I]-Pancreatic Polypeptide binding: 3.7 pmol/mg protein

K_d for [¹²⁵I]-Pancreatic Polypeptide binding: ~0.35 nM

Signal:background: 20-fold

TRANSFECTION: Full-length human PPYR1 cDNA encoding Y₄ (Accession Number: NM_005972)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line with no endogenous Y₄ 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 (EMD 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, 1mM CaCl₂, 0.2% BSA filtered and stored at 4°C

Radioligand: [¹²⁵I]-Pancreatic Polypeptide. (Perkin Elmer #: NEX315)

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 20-fold signal:background with [¹²⁵I] labeled Pancreatic Polypeptide at 0.4 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes proteins were 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:**
1. Michel MC *et al.* (1998) XVI. International Union of Pharmacology. Recommendations for the nomenclature of neuropeptide Y, peptide YY and pancreatic polypeptide receptors. *Pharmacol. Rev.* 50: 143-150.
 2. Sainsbury A *et al.* (2002) Y_4 receptor knockout restores fertility in ob/ob mice. *Genes Dev.* 16:1077-1088.
 3. Sainsbury A *et al.* (2006) Y_2Y_4 receptor double knockout protects against obesity due to a high-fat diet or Y_1 receptor deficiency in mice. *Diabetes* 55: 19-26.

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