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CHEMISCREEN™ MEMBRANE PREPARATION HUMAN Y_4 NEUROPEPTIDE Y RECEPTOR

CATALOG NUMBER:	HTS204M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION PER VIAL:**	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, Y_1, Y_2, Y_4, Y_5 and Y_6, 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. Y_4 binds preferentially to PP, with significant binding to NPY and PYY (Michel *et al.*, 1998). Y_4 plays a role in control of reproduction, as Y_4-null, ob/ob double knockout mice have increased fertility relative to ob/ob mice with functional Y_4 (Sainsbury *et al.,* 2002). In addition, mice lacking Y_4 display resistance to weight gain induced by high fat diet, and mice lacking both Y_2 and Y_4 are resistant to the enhanced weight gain exhibited by Y_1-null mice fed a high fat diet (Sainsbury *et al.,* 2006). Millipore's Y_4 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 Y_4. The membrane preparations exhibit a Kd of 0.35 nM for [^{125}I]-Pancreatic Polypeptide. With 0.4 nM [^{125}I]-Pancreatic Polypeptide, 5μg/well Y_4 Membrane Prep typically yields greater than 20-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay and GTPγS binding.

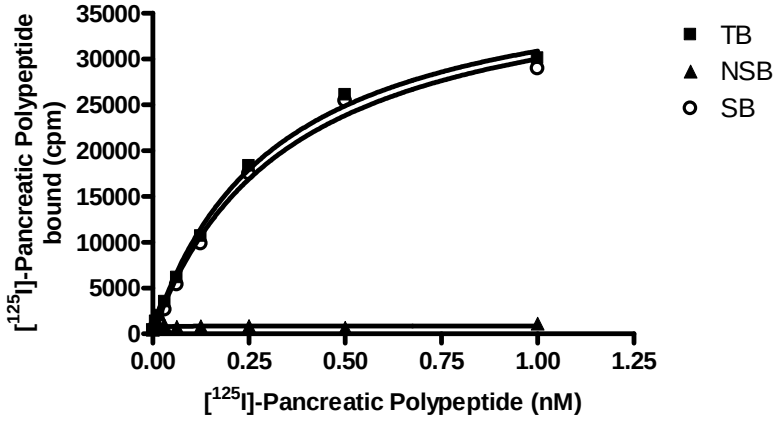

Figure 1. Saturation binding for Y_4. 5 μg/well Y_4 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled Pancreatic Polypeptide in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Pancreatic Polypeptide. Specific binding (SB) was determined by subtracting NSB from TB.

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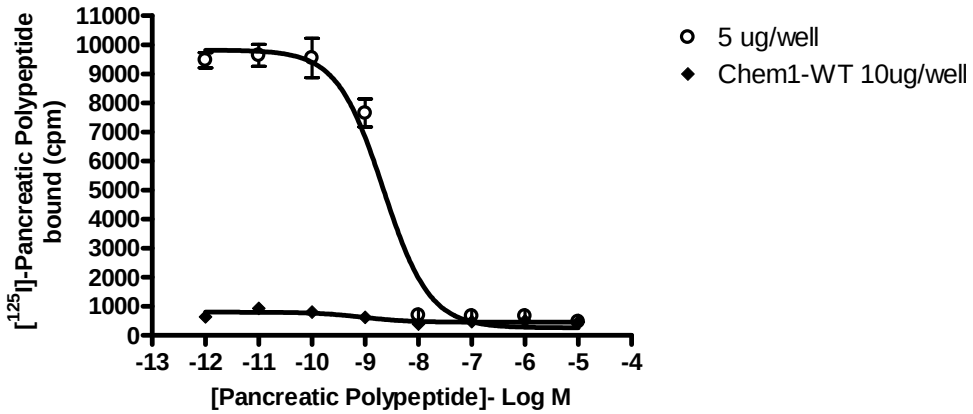

Figure 2. Competition binding for Y_4 . 5μg/well Y_4 Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.4 nM ^{125}I-labeled Pancreatic Polypeptide and increasing concentrations of unlabeled Pancreatic Polypeptide. More than 20-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with 5 μg/well of Y_4 Receptor membrane prep.

	5 μg/well
Signal:background	37.5
Specific binding (cpm)	9558

SPECIFICATIONS: 1 unit = 5 μg
B_{max} for [^{125}I]-Pancreatic Polypeptide binding: 3.7 pmol/mg protein
K_d for [^{125}I]-Pancreatic Polypeptide binding: ~0.35 nM

TRANSFECTION: Full-length human PPYR1 cDNA encoding Y_4 (Accession Number: NM_005972)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous Y_4 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 $MgCl_2$, 1mM CaCl2, 0.2% BSA filtered and stored at 4°C

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

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, 0.1% BSA filtered and stored at 4°C**.**

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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 ^{125}I labeled Pancreatic Polypeptide at 0.4 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 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:

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.

Sainsbury A *et al.* (2002) Y_4 receptor knockout restores fertility in ob/ob mice. *Genes Dev.* 16:1077-1088.

Sainsbury A *et al.* (2006) Y_2 Y_4 receptor double knockout protects against obesity due to a high-fat diet or Y_1 receptor deficiency in mice. *Diabetes* 55: 19-26.

For research use only; not for use as a diagnostic.