

PRODUCT DATASHEET
ChemiScreen™ Y₂ Neuropeptide Y Receptor Membrane Preparation

CATALOG NUMBER:	HTS066M	QUANTITY:	200 units
LOT NUMBER:	22D2904	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 G_{i/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. Y₂ is primarily expressed in the CNS, and it mediates presynaptic inhibition of neurotransmitter release (Michel *et al.*, 1998). The Y₂ 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 Y₂ interactions and its ligands. The membrane preparations exhibit a K_d of 0.104 nM for [¹²⁵I]-human PYY. With 1 nM [¹²⁵I]-human PYY, 5 μg/well Y₂ Membrane Prep yields greater than 10-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay.

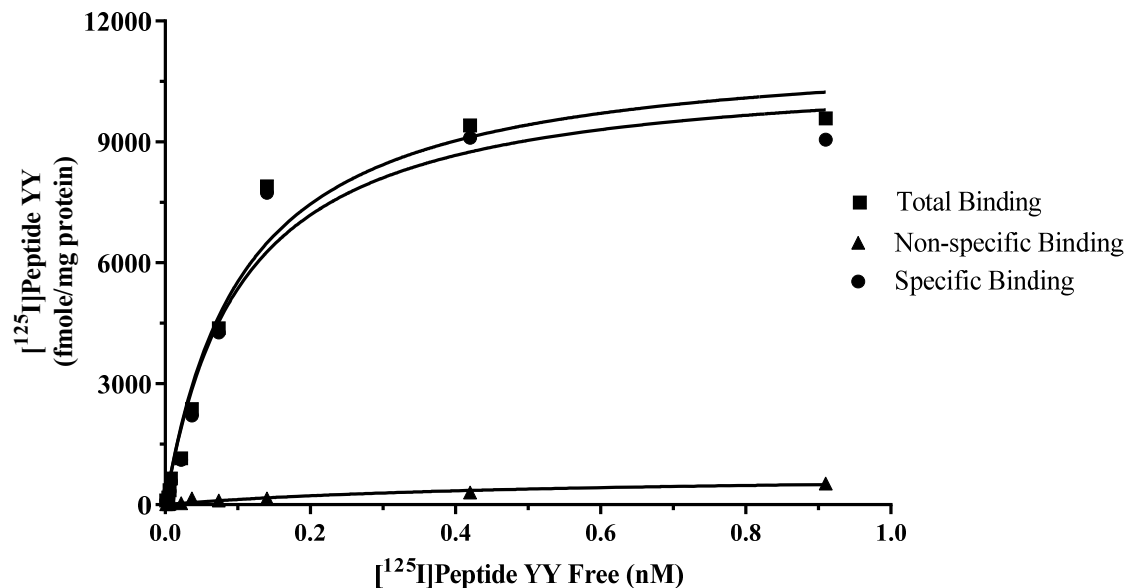


Figure 1. Saturation binding for Y₂. 5 μg/well Y₂ Membrane Preparation was incubated with increasing amount of [¹²⁵I] labeled human PYY in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled NPY. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

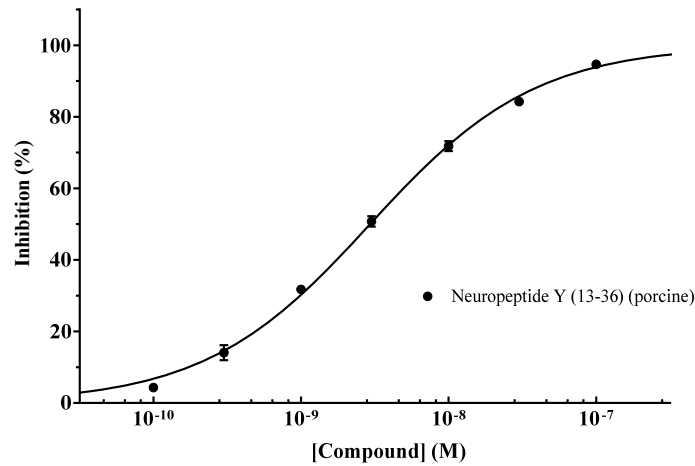


Figure 2. Competition binding for Y₂. Y₂ Membrane Preparation (5 µg/well in a 96-well plate) were incubated with 0.5 nM [¹²⁵I] labeled human PYY and increasing concentrations of unlabeled human PYY, and subjected to filtration binding. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [¹²⁵I] human PYY binding: 10.9 pmol/mg protein
 K_d for [¹²⁵I] human PYY binding: 0.104 nM
 Signal:background: >10-fold

TRANSFECTION: Full-length human NPY2R cDNA encoding Y₂ (Accession Number: NM_000910)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line with no endogenous Y₂ 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]-human PYY (Perkin Elmer#:NEX-341)

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] human PYY.

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.

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