

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN NTR1 NEUROTENSIN RECEPTOR

CATALOG NUMBER: HTS034M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND: Neurotensin is a 13 amino acid peptide that induces hypothermia and mediates analgesia when administered centrally, and also mediates the behavioral effects of antipsychotic drugs in rodents (Kinkead and Nemeroff, 2004). In addition, several intestinal functions, such as contractility and inflammation, are mediated by neurotensin (Castagliuolo *et al.*, 1999). The actions of neurotensin are mediated by two GPCRs, NTR1 (or NTS1) and NTR2 (or NTS2). NTR1 knockout mice are resistant to the hypothermic, analgesic and contractile effects of neurotensin, indicating that NTR1 is the primary mediator of these effects (Pettibone *et al.*, 2002). Small molecule antagonists, including SR48692 and SR142948A, that are partially selective for NTR1 also interfere with the biological effects of neurotensin (Labbé-Jullié *et al.*, 1998).

Chemicon's NTR1 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 neurotensin/NTR1 interactions. The membrane preparations exhibit a K_d of 1.5 nM for [¹²⁵I]-neurotensin. With 5 µg/well NTR1 Membrane Prep and 0.5 nM [¹²⁵I]-neurotensin, a greater than 5-fold signal-to-background ratio is obtained.

APPLICATIONS: Radioligand binding assay, and GTPγS binding.

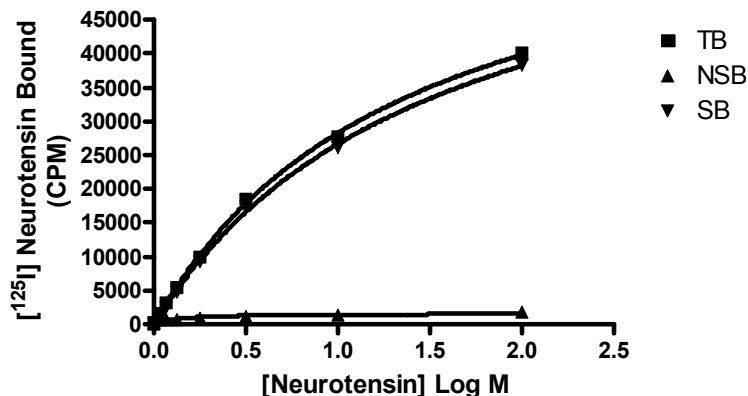


Figure 1. Saturation binding for NTR1. 10 µg/well NTR1 Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled neurotensin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled neurotensin. Specific binding (SB) was determined by subtracting NSB from TB.

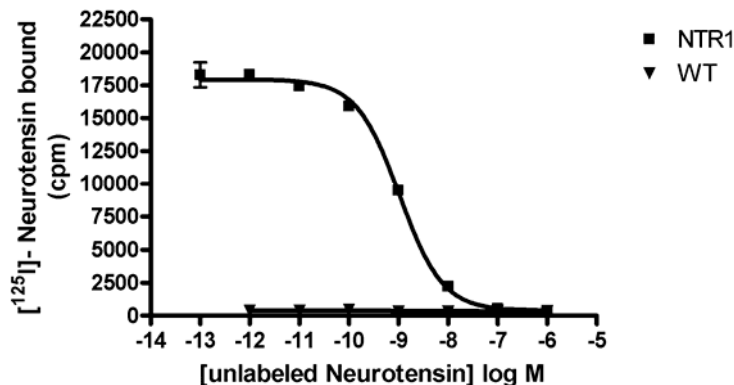


Figure 2. Competition binding for NTR1. 5 μ g/well NTR1 Membrane Preparation (HTS034M) and Wild-Type Chem-2 Membrane Preparation (WT; Chemicon cat. # HTS000MC2) were incubated with 0.5 nM 125 I-labeled neurotensin and increasing concentrations of unlabeled neurotensin. More than 5-fold signal:background was obtained with NTR1 Membrane Preparation, whereas no signal was observed in wild-type Membrane Preparation.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with NTR1 membrane prep.

	5 μ g/well
Signal:background	47
Specific binding (cpm)	17556

SPECIFICATIONS: 1 unit = 5 μ g
 Bmax: 12.9 pmol/mg
 Kd: 1.5 nM

TRANSFECTION: Full length NTSR1 cDNA encoding human NTR1 (Accession number NM_002531)

HOST CELL: Chem-2, a suspension mammalian cell line without any endogenous NTR1 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: 50mM HEPES, 5mM MgCl₂, 1mM CaCl₂, 0.2% BSA, pH to 7.4 and filter.

Radioligand: [125 I] Neurotensin (PerkinElmer#: NEX198)

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

One vial contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 5-fold signal:background with 125 I-labeled neurotensin at 0.5 nM.

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- 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:**
- Castagliuolo I *et al.* (1999) Neurotensin is a proinflammatory neuropeptide in colonic inflammation. *J. Clin. Invest.* 103: 843-849.
- Kinkead B. and Nemeroff C. (2004) Neurotensin, schizophrenia, and antipsychotic drug action. *Int. Rev. Neurobiol.* 59: 327-349.
- Labbé-Jullié C *et al.* (1998) Mutagenesis and modeling of the neurotensin receptor NTR1: Identification of residues that are critical for binding SR48692, a nonpeptide neurotensin antagonist. *J. Biol. Chem.* 273: 16351-16357.
- Pettibone D.J *et al.* (2002) The effects of deleting the mouse neurotensin receptor NTR1 on central and peripheral responses to neurotensin. *J. Pharmacol. Exp. Ther.* 300: 305-313.
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