

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN NK₂ TACHYKININ RECEPTOR

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|------------------------|---------|------------------------------|-----------------|
| CATALOG NUMBER: | HTS144M | QUANTITY: | 200 units |
| LOT NUMBER: | | VOLUME/CONCENTRATION: | 2 mL, 1.0 mg/mL |

BACKGROUND:

The tachykinin peptide family in mammals comprises three peptides, substance P, neurokinin A and neurokinin B, which bind to the tachykinin receptor family of GPCRs, NK₁, NK₂ and NK₃ (Severini *et al.*, 2002). Tachykinins have prominent activity in the GI system, in which they stimulate intestinal contraction and salivation; these effects are mediated by NK₁ and NK₂. Experiments with selective antagonists of NK₂, SR 48,968 and MEN 10,376 indicate that NK₂ mediates neurokinin- induced constriction of pulmonary artery and bronchus (Maggi *et al.*, 1993). NK₂ antagonists display efficacy in treatment of irritable bowel disorder (Lecci *et al.*, 2004). Millipore's NK₂ 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 NK₂ receptor interactions with its ligand. The membrane preparations exhibit a K_d of 0.76nM for [¹²⁵I]-NKA. With 10.0 ug/well NK₂ Membrane Prep and 0.5nM [¹²⁵I]-NKA, a greater than 10-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay, and GTPγS binding.

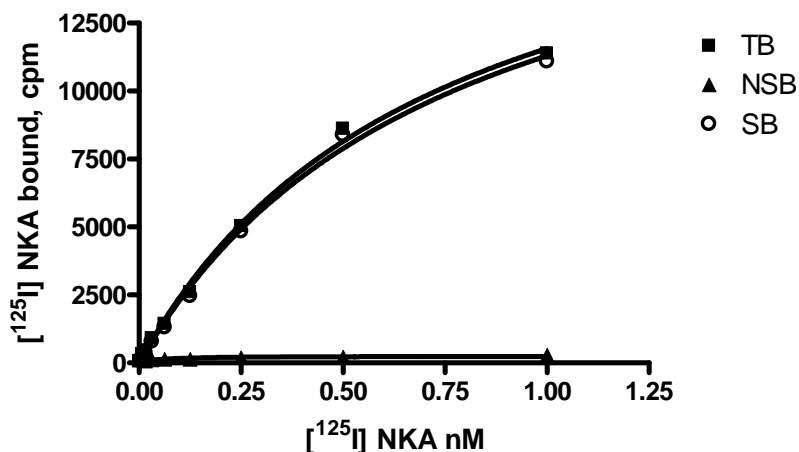


Figure 1. Saturation binding for NK₂ Receptor. 10 μg/well NK₂ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-NKA in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled human recombinant NK₂. Specific binding (SB) was determined by subtracting NSB from TB.

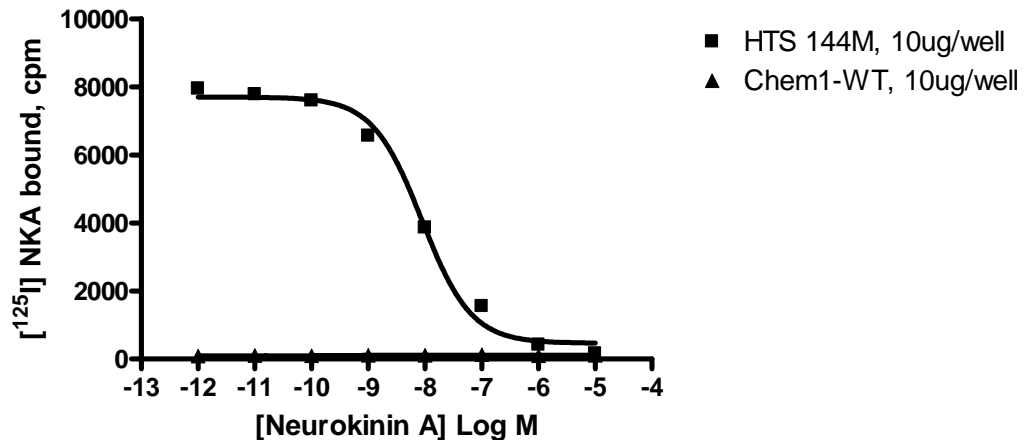


Figure 2. Competition binding for NK₂ Receptor. NK₂ Receptor Membrane Preparation (10µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.5nM [¹²⁵I]-NKA and increasing concentrations of unlabeled Neurokinin A, and more than 10- fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with 10ug/well of NK₂ Receptor membrane prep.

| | |
|------------------------|---------------|
| | 10 µg/well |
| Signal:background | 16.5 |
| Specific binding (cpm) | 19906 |

SPECIFICATIONS: 1 unit = 10.0 µg membrane preparation
 B_{max}: 2.21 pmol/mg
 K_d: 0.76 nM

Species: Full-length human TACR2 cDNA encoding NK₂ (Accession number NM_001057)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous NK₂ 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 Tris, pH 7.4. 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 Tris, pH 7.4, 150mM NaCl, 3mM MnCl₂, 0.1% BSA, containing one Protease Inhibitor Cocktail Tablet (Roche Cat. No. 11 873 580 001) for each 50ml binding buffer.

Radioligand: [¹²⁵I]-NKA (NEX 252)

Wash Buffer: 50mM Tris, pH 7.4, 150mM NaCl, 3mM MnCl, 0.1% BSA

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One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 10-fold signal:background with ¹²⁵I-labeled NKA at 0.5nM.

- PRESENTATION:** Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membranes protein was 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:** Lecci A *et al.* (2004) Tachykinin NK2 receptor antagonists for the treatment of irritable bowel syndrome. *Br. J. Pharmacol.* 141: 1249-63.
- Maggi CA *et al.* (1993) In vivo and in vitro pharmacology of SR 48,968, a non-peptide tachykinin NK2 receptor antagonist. *Eur. J. Pharmacol.* 234: 83-90.
- Severini C *et al.* (2002) The Tachykinin Peptide Family. *Pharmacol. Rev.* 54: 285-322

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