eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ NK_1 TACHYKININ Membrane Preparation

CATALOG NUMBER:	HTS080M	**QUANTITY:**	200 units
LOT NUMBER:	21E1807	**VOLUME/CONCENTRATION:**	1 mL, 2.0 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, and an antagonist of NK1, aprepitant, is used for treatment of chemotherapy-induced emesis (Rupniak and Kramer, 1999). The NK1 tachykinin receptor is expressed in brain, and is thought to be involved in depression and nociception (Saria, 1999). Although NK1 appears to promote nociception in animal models, the significance of this activity is controversial, as antagonists of NK1 have not proven efficacious in pain relief in humans (Hill, 2000). EMD Millipore's NK_1 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 NK_1 interactions with Substance P. The membrane preparations exhibit a Kd of 0.28-0.4 nM for [^{125}I]-Substance P. With 10 µg/well NK_1 Membrane Prep and 0.25 nM [^{125}I]-Substance P, a greater than 5-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand Binding Assay

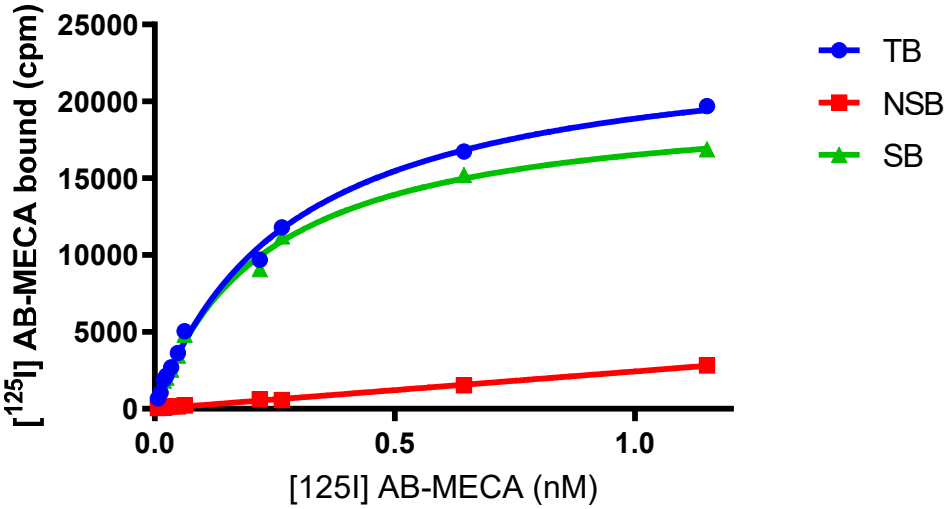

Figure 1. Saturation binding for NK_1. 10 µg/well NK_1 Membrane Preparation was incubated with increasing amount of [^{125}I]-Substance P in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled 1 µM [Sar^9,$Met(O_2)^{11}$]-substance P.. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

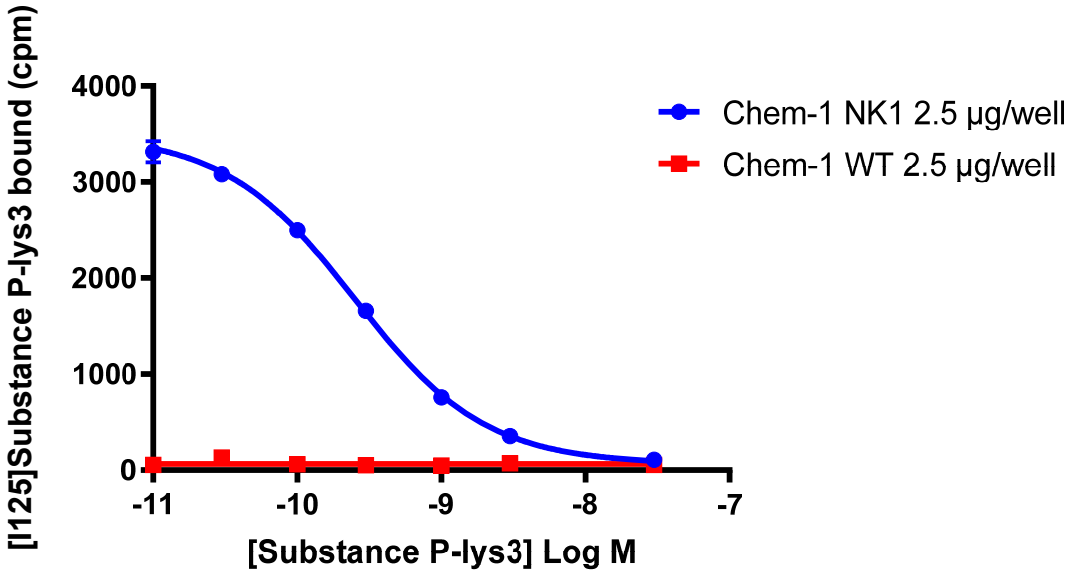

Figure 2. Competition binding for NK_1. NK_1 Membrane Preparation (10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.25 nM [^{125}I]-Substance P and increasing concentrations of unlabeled [Sar^{9},Met(O_2)^{11}]-substance P, and more than 5-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{3}H] SCH-23390 binding: 1.8 pmol/mg protein
K_d for [^{3}H] SCH-23390 binding: 0.13 nM
Signal:background: ≥5-fold

Species: Full-length human TACR1 cDNA encoding NK_1 (Accession Number: NM_001058)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous D_1 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 50 mM 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 Tris-HCl (pH 7.4), 5 mM MnCl2, 0.2 % BSA and 40 µg/ml bacitracin, filtered and stored at 4°C.

Radioligand: [^{125}I] Substance P (Perkin Elmer # NEX190)

Wash Buffer: 50 mM Tris, pH 7.4 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 [^{125}I] Substance P at 0.05 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml

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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Missale C *et al.* (1998). Dopamine receptors: from structure to function. *Physiol. Rev.* 78:189-225.