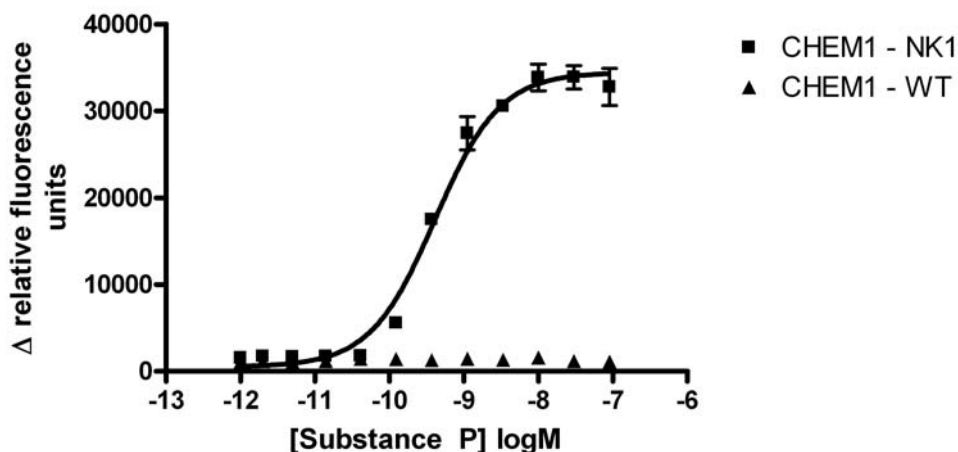


**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT NK<sub>1</sub> TACHYKININ RECEPTOR**

<b>CATALOG NUMBER:</b>	HTS080C	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/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, NK<sub>1</sub>, NK<sub>2</sub> and NK<sub>3</sub> (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 NK<sub>1</sub> and NK<sub>2</sub>, and an antagonist of NK<sub>1</sub>, aprepitant, is used for treatment of chemotherapy-induced emesis (Rupniak and Kramer, 1999). The NK<sub>1</sub> tachykinin receptor is expressed in brain, and is thought to be involved in depression and nociception (Saria, 1999). Although NK<sub>1</sub> appears to promote nociception in animal models, the significance of this activity is controversial, as antagonists of NK<sub>1</sub> have not proven efficacious in pain relief in humans (Hill, 2000). Chemicon's cloned human NK<sub>1</sub>-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant NK<sub>1</sub> expression on the cell surface and contains high levels of the promiscuous G protein G<sub>α15</sub> to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between NK<sub>1</sub> and its ligands.

**APPLICATIONS:** Calcium flux assay, ligand binding assays



**Figure 1.** Calcium flux in NK<sub>1</sub>-expressing Chem-1 cell line induced by Substance P analog. NK<sub>1</sub>-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-Substance P (10<sup>-7</sup> to 10<sup>-12</sup> M) was determined in triplicate on a Molecular Devices Flex Station. An increase of greater than 34,000 fluorescence units was obtained for Substance P.

**SPECIFICATIONS:** EC<sub>50</sub> for calcium mobilization by [Sar<sup>9</sup>,Met(O<sub>2</sub>)<sup>11</sup>]-Substance P: ~ 0.41 nM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein,  $G_{\alpha 15}$ .

TRANSFECTION: Full-length human TACR1 cDNA encoding NK<sub>1</sub> (Accession Number: NM\_001058)

GROWTH MEDIA: DMEM containing 4.5 g/L glucose/10% heat inactivated fetal bovine serum/1x nonessential amino acids/10 mM HEPES/0.25 mg/ml Geneticin (G418)/100 U/ml each penicillin and streptomycin

**PRESENTATION:**

Cells are frozen at  $2 \times 10^6$  cells/mL in Cell Culture Freezing Media with DMSO (Chemicon catalog number S-002-10F). Cell line tests negative for mycoplasma.

**STORAGE/HANDLING:**

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing 20 mL growth media, and place in a humidified 37°C incubator with 5% CO<sub>2</sub>. After 8-24 h, cells will adhere to the plate, at which time the media should be replaced to remove residual DMSO. Cells are passaged by washing with Ca<sup>++</sup> and Mg<sup>++</sup>-free HBSS (10 mL/T75), incubating with 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) for 5-10 minutes at 37°C, and rapping the side of the flask to dislodge the cells. Neutralize the trypsin by addition of 4 volumes growth media. Cells are typically passaged 1:10 with every 3-4 days, and should be passaged at least once after thawing prior to use in calcium flux assays.

**REFERENCES:**

Hill R (2000) NK<sub>1</sub> (substance P) receptor antagonists--why are they not analgesic in humans? *Trends Pharmacol. Sci.* 21: 244-246.

Rupniak NM and Kramer MS (1999) Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK<sub>1</sub>) antagonists. *Trends Pharmacol. Sci.* 20: 485-490.

Saria A (1999) The tachykinin NK<sub>1</sub> receptor in the brain: pharmacology and putative functions. *Eur. J. Pharmacol.* 375: 51-60.

Severini C *et al.* (2002) The Tachykinin Peptide Family. *Pharmacol. Rev.* 54: 285-322.

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HUMAN RECOMBINANT NK<sub>1</sub> TACHYKININ RECEPTOR**

**Product No. HTS080C**

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