

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT NMU1 NEUROMEDIN U RECEPTOR**

CATALOG NUMBER: HTS062C **QUANTITY:** 2 vials, 1 mL per vial
LOT NUMBER: **CONCENTRATION:** 2 x 10⁶ cells/mL

BACKGROUND: Neuromedin U (NmU) is a peptide characterized by its ability to promote smooth muscle contraction (Brighton *et al.*, 2004a). Two GPCRs, NMU1 and NMU2, mediate the contractile effects of neuromedin U by activation of both G_q and G_i (Brighton *et al.*, 2004b). In addition, NmU binds to NMU1 expressed on Th2 cells to induce cytokine release (Johnson *et al.*, 2004). Chemicon's cloned human NMU1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant NMU1 expression on the cell surface and contains high levels of the promiscuous G protein G_α15 to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between NMU1 and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

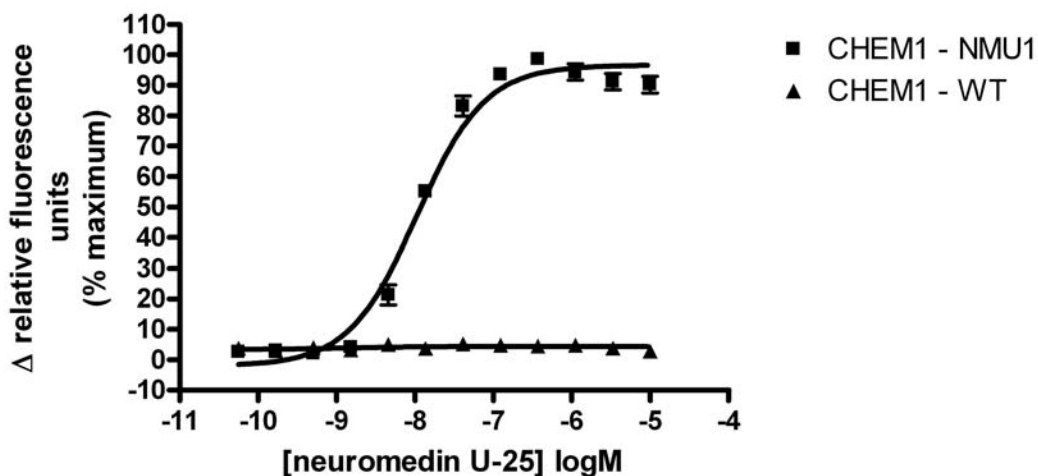


Figure 1. Calcium flux in NMU1-expressing Chem-1 cell line induced by Neuromedin U. NMU1-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 NW and calcium flux in response to Neuromedin U (10⁻⁵ to 10^{-10.5} M) was determined in triplicate on a Molecular Devices FLIPR TETRA.

SPECIFICATIONS: EC50 for calcium mobilization by Neuromedin U: ~ 10.6 nM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G_α15.

TRANSFECTION: Full-length human NMUR1 cDNA encoding NMU1 (Accession Number: NM_006056)

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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 2×10^6 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. 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% CO₂. 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⁺⁺ and Mg⁺⁺-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:** Brighton PJ *et al.* (2004a) Neuromedin U and its receptors: structure, function, and physiological roles. *Pharmacol. Rev.* 56: 231-48
- Brighton PJ *et al.* (2004b) Signaling and ligand binding by recombinant neuromedin U receptors: evidence for dual coupling to Galphaq/11 and GalphaI and an irreversible ligand-receptor interaction. *Mol. Pharmacol.* 66: 1544-56.
- Johnson EN *et al.* (2004) Neuromedin U elicits cytokine release in murine Th2-type T cell clone D10.G4.1. *J. Immunol.* 173(12):7230-8

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HUMAN RECOMBINANT NMU1 NEUROMEDIN U RECEPTOR**

Product No. HTS062C

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