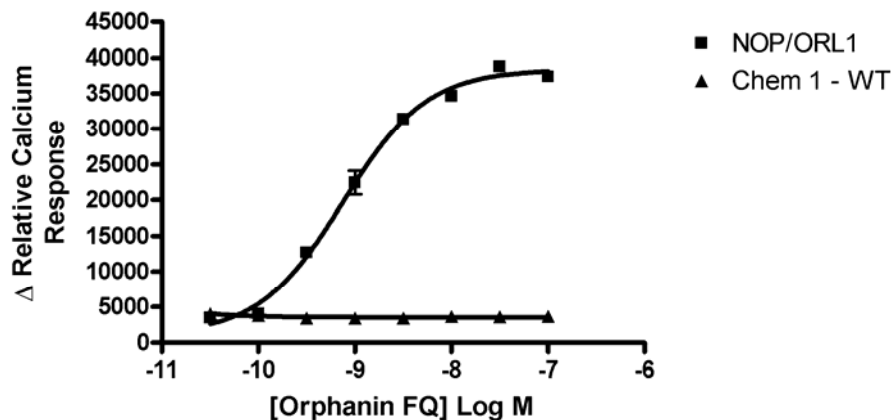


**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT NOP<sub>1</sub>/ORL1 OPIOID RECEPTOR**

<b>CATALOG NUMBER:</b>	HTS040C	<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 NOP<sub>1</sub> receptor (also known as ORL1) is related to the opioid receptor family of GPCRs but does not bind to classical opioids. An endogenous ligand for NOP<sub>1</sub> has been characterized and termed orphanin FQ or nociceptin (OFQ/N), which in turn does not bind to other members of the opioid receptor family. NOP<sub>1</sub> is expressed widely in the CNS, and binding of OFQ/N to NOP<sub>1</sub> appears to function in nociception, locomotor activity, anxiety, reward, memory and tolerance to classical opioids (Mogil and Pasternak, 2001). Chemicon's cloned human NOP<sub>1</sub>/ORL1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant NOP<sub>1</sub>/ORL1 expression on the cell surface and contains high levels of the promiscuous G protein G<sub>α</sub>15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between NOP<sub>1</sub>/ORL1 and its ligands.

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



**Figure 1.** Calcium flux in NOP/ORL1-expressing Chem-1 cell line induced by Orphanin FQ. NOP/ORL1-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 recombinant human Orphanin FQ (10<sup>-7</sup> to 10<sup>-10.5</sup> M) was determined in triplicate on a Molecular Devices Flex Station. Greater than 35,000 fluorescence units above baseline were obtained.

**SPECIFICATIONS:** EC50 for calcium mobilization by Orphanin FQ/nociceptin: ~ 0.7 nM

**HOST CELLS:** Chem-1, an adherent cell line expressing the promiscuous G-protein, G<sub>α</sub>15.

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TRANSFECTION: Full-length human NOP<sub>1</sub>/ORL1 cDNA (Accession Number: X72304)

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 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% 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:** Mogil J.S. and Pasternak G.W. (2001) The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol. Rev.* 53: 381-415.

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**Product No. HTS040C**

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