eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ NOP_1/ORL1 Opoid Receptor Stable Cell Line

CATALOG NUMBER: HTS040C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

The NOP_1 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_1 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_1 is expressed widely in the CNS, and binding of OFQ/N to NOP_1 appears to function in nociception, locomotor activity, anxiety, reward, memory and tolerance to classical opioids (Mogil and Pasternak, 2001). The cloned human NOP_1/ORL1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant NOP_1/ORL1 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 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_1/ORL1 and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

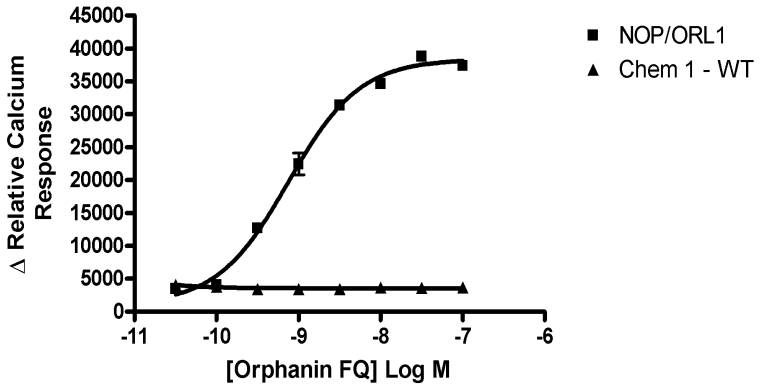

Figure 1. Representative data for activation of the NOP_1/ORL1 receptor stably expressed in Chem-1 cells induced by Orphanin FQ using a fluorescent calcium flux assay. NOP_1/ORL1–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of NOP_1/ORL1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Orphanin FQ	Calcium Flux - Fluorescence	0.7	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.40	72

ASSAY SETUP

Fluorescence

Table 4. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Orphanin FQ ligand	Sigma: O4011
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human NOP_1/ORL1 cDNA (Accession Number: NM_000913; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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                                                    ATG GAG CCC CTC TTC CCC      60
                                                     M   E   P   L   F   P      20

GCG CCG TTC TGG GAG GTT ATC TAC GGC AGC CAC CTT CAG GGC AAC CTG TCC CTC CTG AGC     120
 A   P   F   W   E   V   I   Y   G   S   H   L   Q   G   N   L   S   L   L   S      40

CCC AAC CAC AGT CTG CTG CCC CCG CAT CTG CTG CTC AAT GCC AGC CAC GGC GCC TTC CTG     180
 P   N   H   S   L   L   P   P   H   L   L   L   N   A   S   H   G   A   F   L      60

CCC CTC GGG CTC AAG GTC ACC ATC GTG GGG CTC TAC CTG GCC GTG TGT GTC GGA GGG CTC     240
 P   L   G   L   K   V   T   I   V   G   L   Y   L   A   V   C   V   G   G   L      80

CTG GGG AAC TGC CTT GTC ATG TAC GTC ATC CTC AGG CAC ACC AAA ATG AAG ACA GCC ACC     300
 L   G   N   C   L   V   M   Y   V   I   L   R   H   T   K   M   K   T   A   T     100

AAT ATT TAC ATC TTT AAC CTG GCC CTG GCC GAC ACT CTG GTC CTG CTG ACG CTG CCC TTC     360
 N   I   Y   I   F   N   L   A   L   A   D   T   L   V   L   L   T   L   P   F     120

CAG GGC ACG GAC ATC CTC CTG GGC TTC TGG CCG TTT GGG AAT GCG CTG TGC AAG ACA GTC     420
 Q   G   T   D   I   L   L   G   F   W   P   F   G   N   A   L   C   K   T   V     140

ATT GCC ATT GAC TAC TAC AAC ATG TTC ACC AGC ACC TTC ACC CTA ACT GCC ATG AGT GTG     480
 I   A   I   D   Y   Y   N   M   F   T   S   T   F   T   L   T   A   M   S   V     160

GAT CGC TAT GTA GCC ATC TGC CAC CCC ATC CGT GCC CTC GAC GTC CGC ACG TCC AGC AAA     540
 D   R   Y   V   A   I   C   H   P   I   R   A   L   D   V   R   T   S   S   K     180

GCC CAG GCT GTC AAT GTG GCC ATC TGG GCC CTG GCC TCT GTT GTC GGT GTT CCC GTT GCC     600
 A   Q   A   V   N   V   A   I   W   A   L   A   S   V   V   G   V   P   V   A     200

ATC ATG GGC TCG GCA CAG GTC GAG GAT GAA GAG ATC GAG TGC CTG GTG GAG ATC CCT ACC     660
 I   M   G   S   A   Q   V   E   D   E   E   I   E   C   L   V   E   I   P   T     220

CCT CAG GAT TAC TGG GGC CCG GTG TTT GCC ATC TGC ATC TTC CTC TTC TCC TTC ATC GTC     720
 P   Q   D   Y   W   G   P   V   F   A   I   C   I   F   L   F   S   F   I   V     240

CCC GTG CTC GTC ATC TCT GTC TGC TAC AGC CTC ATG ATC CGG CGG CTC CGT GGA GTC CGC     780
 P   V   L   V   I   S   V   C   Y   S   L   M   I   R   R   L   R   G   V   R     260

CTG CTC TCG GGC TCC CGA GAG AAG GAC CGG AAC CTG CGG CGC ATC ACT CGG CTG GTG CTG     840
 L   L   S   G   S   R   E   K   D   R   N   L   R   R   I   T   R   L   V   L     280

GTG GTA GTG GCT GTG TTC GTG GGC TGC TGG ACG CCT GTC CAG GTC TTC GTG CTG GCC CAA     900
 V   V   V   A   V   F   V   G   C   W   T   P   V   Q   V   F   V   L   A   Q     300

GGG CTG GGG GTT CAG CCG AGC AGC GAG ACT GCC GTG GCC ATT CTG CGC TTC TGC ACG GCC     960
 G   L   G   V   Q   P   S   S   E   T   A   V   A   I   L   R   F   C   T   A     320

CTG GGC TAC GTC AAC AGC TGC CTC AAC CCC ATC CTC TAC GCC TTC CTG GAT GAG AAC TTC    1020
 L   G   Y   V   N   S   C   L   N   P   I   L   Y   A   F   L   D   E   N   F     340

AAG GCC TGC TTC CGC AAG TTC TGC TGT GCA TCT GCC CTG CGC CGG GAC GTG CAG GTG TCT    1080
 K   A   C   F   R   K   F   C   C   A   S   A   L   R   R   D   V   Q   V   S     360
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GAC CGC GTG CGC AGC ATT GCC AAG GAC GTG GCC CTG GCC TGC AAG ACC TCT GAG ACG GTA   1140
 D   R   V   R   S   I   A   K   D   V   A   L   A   C   K   T   S   E   T   V    380

CCG CGG CCC GCA TGA
 P   R   P   A  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS040M	ChemiScreen™ NOP_1/ORL1 Opoid Receptor Membrane Prep

REFERENCES

1. 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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