

#### PRODUCT DATASHEET

# Ready-to-Assay™ NOP₁ Opioid Receptor Frozen Cells

**CATALOG NUMBER: HTS040RTA** 

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N<sub>2</sub>. Media Component at 4°C (-20°C for prolonged storage).

#### **BACKGROUND**

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

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). Cloned human NOP $_1$ -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant NOP $_1$  expression on the cell surface and contains high levels of the promiscuous G protein G $\alpha$ 15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at NOP $_1$ .

#### USE RESTRICTIONS

Please see User Agreement (Label License) for further details. One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.

#### 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.



#### **APPLICATIONS**

Calcium Flux Assays

#### **APPLICATION DATA**

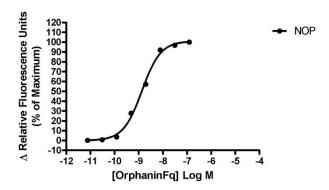


Figure 1. Representative data for activation of NOP<sub>1</sub> receptor. Calcium flux in NOP<sub>1</sub>–expressing Chem-1 cell line induced by OrphaninFq. NOP<sub>1</sub>–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR<sup>TETRA</sup>. Maximal fluorescence signal obtained in this experiment was 3,000 RLU (Relative Light Units).

Table 1. EC<sub>50</sub> value of NOP<sub>1</sub>-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
OrphaninFq	Calcium Flux	1.3	Eurofins Internal Data

#### **ASSAY SETUP**

- 1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
- 2. 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.
- 3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
- 4. Centrifuge the cell suspension at 190 x g for four minutes
- 5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
- 6. Seed cell suspension into appropriate assay microplate (100 μL/well for 96-well plate, 25 μL/well for 384-well plate).
- 7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
- 8. Move assay plate to a humidified 37°C 5% CO2 incubator for 24 hours.
- After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.



- 10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca<sup>2+</sup> dye by dissolving 1mg of Fluo-8 NW in 200 μL of DMSO. Once dissolved place 10 μL of Fluo-8 NW Ca<sup>2+</sup> dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca<sup>2+</sup> dye at 10 μL /10 mL is sufficient for loading one (1) microplate).
- 11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR<sup>TETRA</sup>) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR<sup>TETRA</sup>) or emission filter for Ca<sup>2+</sup> dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 μL below liquid level and dispense rate to 75 μL/sec (96-well format) or 50 μL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
- 12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 96-well or Corning 3574 384-well).
- 13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

#### **ASSAY MATERIALS**

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
OrphaninFq ligand	Sigma: O4011
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

#### **FLIPR SETTINGS**

Settings for FLIPR<sup>TETRA</sup>® 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	25 μl (50 μl for 384-well)
Dispense Speed	75 μl L/sec (50 μl for 384-well)
Expel Volume	0 μΙ
Analysis	Subtract Bias Sample 1

#### **HOST CELL**

Chem-1, an adherent rat hematopoietic cell line expressing endogenous  $G\alpha 15$  protein.

#### **EXONGENOUS GENE EXPRESSION**

ORL1 cDNA (Accession Number: NM\_000913; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.



### **CODING SEQUENCE**

														ATG M	GAG E	CCC P	CTC L	TTC F	CCC P	60 20
GCG A	CCG P	TTC F	TGG W	GAG E	GTT V	ATC I	TAC Y	GGC G	AGC S	CAC H	CTT L	CAG Q	GGC G	AAC N	CTG L	TCC S	CTC L	CTG L	AGC S	120 40
CCC P	AAC N	CAC H	AGT S	CTG L	CTG L	CCC P	CCG P	CAT H	CTG L	CTG L	CTC L	AAT N	GCC A	AGC S	CAC H	GGC G	GCC A	TTC F	CTG L	180 60
CCC P	CTC L	GGG G	CTC L	AAG K	GTC V	ACC T	ATC I	GTG V	GGG G	CTC L	TAC Y	CTG L	GCC A	GTG V	TGT C	GTC V	GGA G	GGG G	CTC L	240 80
CTG L	GGG G	AAC N	TGC C	CTT L	GTC V	ATG M	TAC Y	GTC V	ATC I	CTC L	AGG R	CAC H	ACC T	AAA K	ATG M	AAG K	ACA T	GCC A	ACC T	300 100
AAT N	ATT I	TAC Y	ATC I	TTT F	AAC N	CTG L	GCC A	CTG L	GCC A	GAC D	ACT T	CTG L	GTC V	CTG L	CTG L	ACG T	CTG L	CCC P	TTC F	360 120
CAG Q	GGC G	ACG T	GAC D	ATC I	CTC L	CTG L	GGC G	TTC F	TGG W	CCG P	TTT F	GGG G	AAT N	GCG A	CTG L	TGC C	AAG K	ACA T	GTC V	420 140
ATT	GCC A	ATT	GAC D	TAC Y	TAC Y	AAC N	ATG M	TTC F	ACC T	AGC S	ACC T	TTC F	ACC T	CTA L	ACT T	GCC A	ATG M	AGT S	GTG V	480 160
GAT D	CGC R	TAT Y	GTA V	GCC A	ATC I	TGC C	CAC H	CCC P	ATC I	CGT R	GCC A	CTC L	GAC D	GTC V	CGC R	ACG T	TCC S	AGC S	AAA K	540 180
GCC A	CAG Q	GCT A	GTC V	AAT N	GTG V	GCC A	ATC I	TGG W	GCC A	CTG L	GCC A	TCT S	GTT V	GTC V	GGT G	GTT V	CCC P	GTT V	GCC A	600 200
ATC I	ATG M	GGC G	TCG S	GCA A	CAG Q	GTC V	GAG E	GAT D	GAA E	GAG E	ATC I	GAG E	TGC C	CTG L	GTG V	GAG E	ATC I	CCT P	ACC T	660 220
I	М	G	S	А	Q	V	E	D	Ε	E	I	E	С	L	V	E		P	Т	660 220 720 240
I CCT P	M CAG Q	G GAT D	S TAC Y	A TGG W	Q GGC G	V CCG P	E GTG V	D TTT F	E GCC A	E ATC I	I TGC C	E ATC I	C TTC F	L CTC L	V TTC F	E TCC S	I TTC	P ATC I	T GTC V	220 720
I CCT P CCC P	M CAG Q GTG V	G GAT D CTC L	S TAC Y GTC V	TGG W ATC I	Q GGC G TCT S	V CCG P GTC V	E GTG V TGC C	D TTT F TAC Y	E GCC A AGC S	E ATC I CTC L	I TGC C ATG M	E ATC I ATC I	C TTC F CGG R	L CTC L CGG R	V TTC F CTC L	E TCC S CGT R	I TTC F GGA	P ATC I GTC V	T GTC V CGC R	220 720 240 780
I CCT P CCC P CTG L	M CAG Q GTG V CTC L	G GAT D CTC L TCG S	S TAC Y GTC V GGC G	TGG W ATC I TCC S	Q GGC G TCT S CGA R	V CCG P GTC V GAG E	E GTG V TGC C	D TTT F TAC Y GAC D	E GCC A AGC S CGG R	E ATC I CTC L AAC N	I TGC C ATG M CTG L	E ATC I ATC I CGG R	C TTC F CGG R CGC R	L CTC L CGG R ATC I	V TTC F CTC L ACT T	TCC S CGT R CGG R	I TTC F GGA G	P ATC I GTC V GTG V	T GTC V CGC R CTG L	720 240 780 260 840
I CCT P CCC P CTG L GTG V	M CAG Q GTG V CTC L GTA V	GGAT D CTC L TCG S GTG V	S TAC Y GTC V GGC G GCT A	TGG W ATC I TCC S GTG V	Q GGC G TCT S CGA R TTC F	V CCG P GTC V GAG E GTG V	E GTG V TGC C AAG K GGC G	D TTT F TAC Y GAC D TGC C	E GCC A AGC S CGG R TGG W	E ATC I CTC L AAC N ACG T	TGC C ATG M CTG L CCT P	E ATC I ATC I CGG R GTC V	C TTC F CGG R CGC R	L CTC L CGG R ATC I GTC V	V TTC F CTC L ACT T TTC F	E TCC S CGT R CGG R CGG V	TTC F GGA G CTG L CTG	P ATC I GTC V GTG V GCC A	T GTC V CGC R CTG L CAA Q	220 720 240 780 260 840 280 900
I CCT P CCC P CTG L GTG V GGG G CTG	M CAG Q GTG V CTC L GTA V CTG L GGC	GAT D CTC L TCG S GTG V GGG G TAC	S TAC Y GTC V GGC G GCT A GTT V GTC	TGG W  ATC I  TCC S  GTG V  CAG Q  AAC	Q GGC G TCT S CGA R TTC F CCG P	V CCG P GTC V GAG E GTG V AGC S TGC	E GTG V TGC C AAG K GGC G AGC S CTC	D TTT F TAC Y GAC D TGC C GAG E AAC	E GCC A AGC S CGG R TGG W ACT T CCC	E ATC I CTC L AAC N ACG T GCC A ATC	TGC C ATG M CTG L CCT P GTG V CTC	E ATC I ATC I CGG R GTC V GCC A TAC	C TTC F CGG R CGC R CAG Q ATT I GCC	L CTC L CGG R ATC I GTC V CTG L TTC	V TTC F CTC L ACT T TTC F CGC R CTG	E TCC S CGT R CGG R GTG V TTC F GAT	I TTC F GGA G CTG L CTG L	P ATC I GTC V GTG V GCC A ACG T AACC	T GTC V CGC R CTG L CAA Q GCC A TTC	220 720 240 780 260 840 280 900 300
I CCT P CCC P CTG L GTG V GGG G CTG L	M CAG Q GTG V CTC L GTA V CTG G G G G G G G G G G G G G G G G G G	GGTG V GGG G TAC Y TGC	S TAC Y GTC V GGC G GCT A GTT V GTC V TTC	A TGG W ATC I TCC S GTG V CAG Q AAC N	Q GGC G TCT S CGA R TTC F CCG P	V CCG P GTC V GAG E GTG V AGC S TGC C TTC	E GTG V TGC C AAG K GGC G AGC S CTC L	TTTT F  TAC Y  GAC D  TGC C  GAG E  AAC N  TGT	E GCC A AGC S CGG R TGG W ACT T CCC P GCA	E ATC I CTC L AAC N ACG T GCC A ATC I TCT	TGC C C ATG M CTG L CCT P GTG V CTC L GCC	ATC I ATC I CGG R GTC V GCC A TAC Y CTG	C TTC F CGG R CGC A CGC A CGC	CTC L CGG R ATC I GTC V CTG L TTC F CGG	V TTC F CTC L ACT T TTC F CGC R CTG L	E TCC S CGT R CGG R TTC F GAT D GTG	TTC F GGA C C GAG E CAG	PATC I GTC V GTG V GCC A ACG T AACC N GTG	T GTC V CGC R CTG L CAA Q GCC A TTC F TCT	220 720 240 780 260 840 280 900 300 960 320
I CCT P CCC P CTG L GTG V GGG G CTG L AAG K GAC	M CAG Q GTG V CTC L GTA V CTG L GGC G GCC A CGC	G GAT D CTC L TCG S GTG V GGG G TAC Y TGC C GTG	S TAC Y GTC V GGC G GCT A GTT V GTC V TTC F	A TGG W  ATC I TCC S GTG V  CAG Q  AAC N  CGC R	Q GGC G TCT S S CGA R TTC F CCG P AGC S AAG K ATT	V CCG P GTC V GAG E GTG V AGC S TTC C GCC	E GTG V TGC C AAG K GGC G AGC TC L TGC C AAG	D TTT F TAC Y GAC D TGC C GAG E AAC N TGT C	E GCC A AGC S CGG R TGG W ACT T CCC P GCA A GTG	E ATC I CTC L AACC N ACG T GCC A ATC I TCT S GCC	TGC C ATG M CTG L CCT P GTG V CTC L GCC A	E ATC I ATC I CGG R GTC V GCC A TAC Y CTG L GCC	C TTC F CGG R CAG Q ATT I GCC A CGC R TGC	L CTC L CGG R ATC I GTC V CTG L CGG R AAG	V TTC F CTC L ACT T TTC F CGC R CTG L GAC D ACC	E TCC S CGT R CGG R GTG V TTC F GAT D GTG V TCT	TTC F GGA G CTG L CTG C CGAG E CAG G	P ATC I GTC V GTG V GCC A ACG T AAC N GTG V ACG	T GTC V CGC R CTG L CAA Q GCC A TTC F TCT S GTA	220 720 240 780 260 840 280 900 300 960 320 1020 340



#### **RELATED PRODUCTS**

PRODUCT NUMBER DESCRIPTION

HTSCHEM-1RTA Ready-to-Assay™ Chem-1 host frozen cells (control cells)

HTS040M ChemiScreen™ NOP Opioid 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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