GeneBLAzer® OPRL-1-Gqi5- NFAT-*bla* FreeStyle™ 293F Cells

Catalog Numbers – K1714

Cell Line Descriptions

GeneBLAzer® OPRL-Gqi5-NFAT-*bla* FreeStyle™ 293F cells contain the human opioid/opiate receptor-like-1 (OPRL-1), (Accession #NM_000913) stably integrated into the the GeneBLAzer® Gqi5-NFAT-bla FreeStyle™ 293 Cell Line. GeneBLAzer® Gqi5-NFAT-bla FreeStyle™ 293 cells contain the chimeric Gqi5 G-protein and the beta-lactamase reporter gene under control of the nuclear factor of activated T cells (NFAT) response element.

GeneBLAzer® OPRL-1-Gqi5-NFAT-*bla* FreeStyle™ 293F cells are functionally validated for Z'-factor and EC_{50} concentrations of orphanin FQ/nociceptin (Figure 1). In addition, GeneBLAzer® OPRL-1-Gqi5-NFAT-*bla* FreeStyle™ 293F cells have been tested for assay performance under variable conditions, including DMSO concentration, cell number, stimulation time, and substrate loading time. Additional testing data using alternate stimuli are also included.

Target Description

The opioid (opiate) receptor-like 1 (OPRL1) receptor was initially identified in the screen of a cDNA library from the human brainstem with a DNA probe generated from the PCR of genomic DNA with oligonucleotides created from the conserved regions of opioid receptors (1). OPRL1 is expressed in the central nervous system (2,3), skeletal muscle, vas deferns, intestine, lymphocytes, and spleen (3,4).

Despite 63-65% sequence homology with opioid receptors (OP1, OP2, OP3), OPRL1 does not bind traditional opioid ligands. The endogenous ligand of OPRL1 is a heptadecapeptide identified as Orphanin FQ/Nociceptin (OFQ/N), and does not have high affinity for the opioid receptors (OP1, OP2, OP3) (5,6). The OPRL1 receptor mediates its signaling through the Gαi pathway and is sensitive to pertussis toxin (5,6). In addition to G-protein signaling OFQ/N has also been reported to inhibit voltage-gated calcium channels (7) and stimulate inward rectifying potassium conductance (8).

The functional role of OFQ/N and OPRL1 has been implicated in various physiological functions. The most defined role is its involvement in the mediation of pain in the central nervous system and its possible roles in opiate tolerance, opiate dependence, withdrawl, and the ability to cope with stress and anxiety (9).

Validation Summary

Testing and validation of this assay was evaluated in a 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Orphanin FQ agonist dose response under optimized conditions**

   OrphaninFQ/Nociceptin EC_{50} = 4.16 nM
   Z'-Factor (EC_{100}) = 0.71

   Recommended cell no. = 7.5K cells/well
   DMSO Tolerance = up to 1.0%
   Recommended Stim. Time = 4 hours
   Max. [Stimulation] = 1 µM

2. **Alternate agonist dose response**

   Nociceptin(1-13)NH2 (EC_{50}) = 3.58 nM

3. **Antagonist dose response**

   [Nphe1]-Nocicep.(1-13)NH2 (IC_{50})=78.9 nM
   UFP101 (IC_{50}) = 940 nM

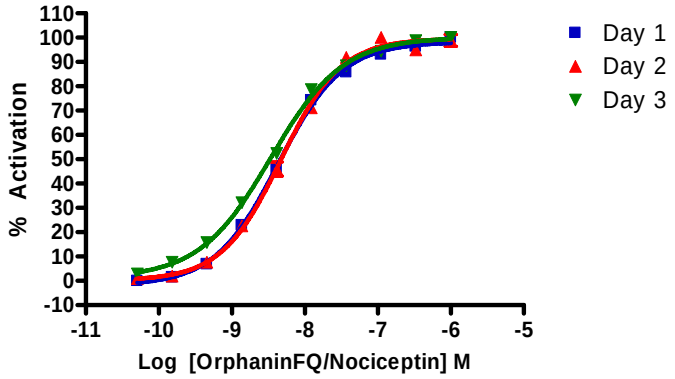
4. **Agonist 2nd messenger dose response**
   Orphanin FQ/Nociceptin (EC_{50}) = 3.1 nM

Assay Testing Summary

5. **Assay performance with variable cell number**
6. **Assay performance with variable stimulation time**
7. **Assay performance with variable substrate loading time**
8. **Assay performance with variable FBS concentration**
9. **Assay performance with variable DMSO concentration**
10. **Assay performance with variable media formulation and % DMSO**

Primary Agonist Dose Response

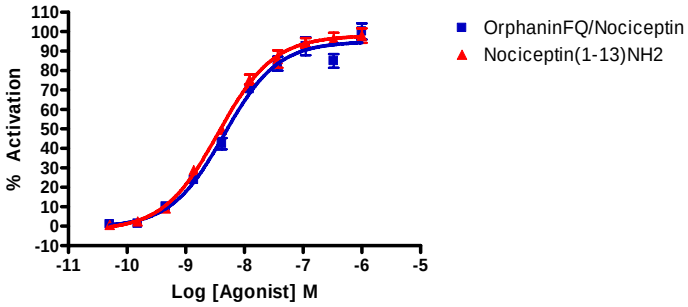
Figure 1 — GeneBLAzer® OPRL-1-Gqi5-NFAT-*bla* FreeStyle™ 293F dose response to Orphanin FQ / nociceptin under optimized conditions



GeneBLAzer® OPRL-1-Gqi5-NFAT-*bla* FreeStyle™ cells (7,500 cells/well) were plated in a 384-well format and incubated for 16-20 hours on 3 separate days. Cells were stimulated with a dilution series of orphanin FQ/nociceptin in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and % Activation plotted for each replicate against the concentrations of orphanin FQ/nociceptin (n=16 for each data point).

Alternate Agonist Dose Response

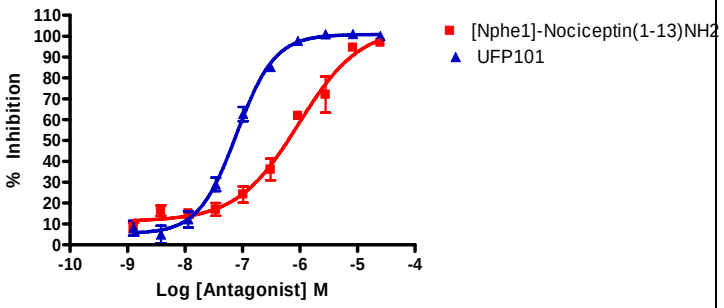
Figure 2 — GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to Orphanin FQ / Nociceptin and Nociceptin (1-13)-NH_2



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ cells were plated at 7,500 cells/well in a black walled, clear-bottom poly-D-lysine 384-well plate and incubated for 16-20 hours. Cells were stimulated with a dilution series of Orphanin FQ/Nociceptin (Sigma #O4011) and Nociceptin (1-13)-NH_2 (Tocris #1358) in the presence of 0.1% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 1 hour. Fluorescence emission values at 460 nm and 530 nm were obtained and the 460/530 ratio is plotted for each cell number against the indicated concentrations of the agonists (n=8 for each data point).

Antagonist Dose Response

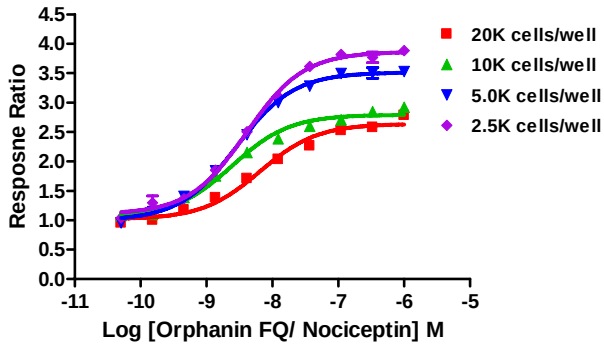
Figure 3 — GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to [Nphe1]-nociceptin (1-13)-NH_2 and UFP-101



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 cells were plated 16-20 hours prior to assay at 7,500 cells per well in a black-walled, clear bottom poly-D-lysine 384-well plate. A dilution series of [Nphe1]-nociceptin (1-13)-NH_2 (Tocris #1308) and UFP-101 (Tocris #1552) in the presence of 0.1% DMSO was added to the cells. The cells were incubated at 37ºC with 5% CO_2 for 30 min. Orphanin FQ/Nociceptin (Sigma #O4011) was added to the plate at the EC_{80} concentration of 17.0 nM. Cells were incubated for 3.5 hours and loaded for 1 hour with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Inhibition shown plotted against the indicated concentrations of the antagonists. (n=8 for each data point).

Assay Performance with Variable Cell Number

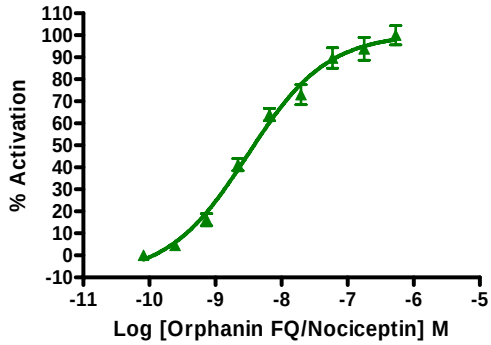
Figure 4 – GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to Orphanin FQ/Nociceptin with 2.5, 5, 10, and 20K cells/well



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 cells were plated at 2500, 5000, 10000 or 20,000 cells/well in a poly-D-lysine treated 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of Orphanin FQ/Nociceptin (Sigma #O4011) in the presence of 0.1% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 1.5 hours. Fluorescence emission values at 460 nm and 530 nm were obtained and the Response Ratios plotted for each cell number against the indicated concentrations of Orphanin FQ/Nociceptin (n=8 for each data point).

Agonist 2nd Messenger Dose Respone

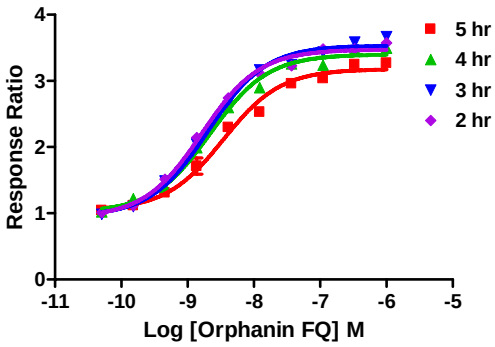
Figure 5 – GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to Orphanin FQ as determined by the measurement of intracellular Ca^{2+} by Fluo-4



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* Freestyle 293F cells were loaded with Fluo4-AM and tested for a response to orphanin FQ/nociceptin.

Assay Performance with Variable Stimulation Time

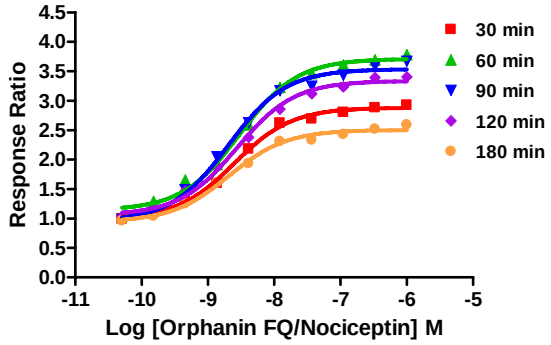
Figure 6 – GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to Orphanin FQ/Nociceptin with 2, 3, 4 and 5 hr. stimulation times



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™293 cells were plated in a poly-D-lysine coated 384-well plate at 5,000 cells/well, and incubated for 16-20 hours. Cells were stimulated with a dilution series of Orphanin FQ/Nociceptin (Sigma #O4011) for 2, 3, 4, or 5 hrs in the presence of 0.1% DMSO. Cells were then loaded for 1.5 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each stimulation time against the indicated concentrations of Orphanin FQ/Nociceptin (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

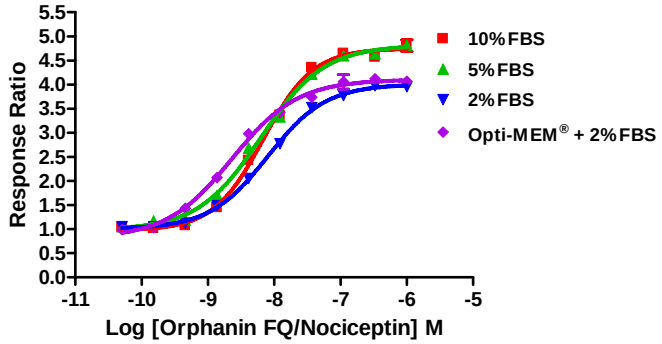
Figure 7 – GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to Orphanin FQ/Nociceptin with 0.5, 1, 1.5, 2, and 3 hour substrate loading times.



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 cells (5,000 cells/well) were plated in a poly-D-lysine 384-well plate and incubated for 16-20 hours. Cells were stimulated with a dilution series of OrphaninFQ/Nociceptin (Sigma #O4011) in the presence of 0.1% DMSO for 3 hours. Cells were then loaded for either 0.5, 1, 1.5, 2, or 3 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each substrate loading time against the indicated concentrations of Orphanin FQ/Nociceptin (n=8 for each data point).

Assay Performance with Variable FBS Concentration

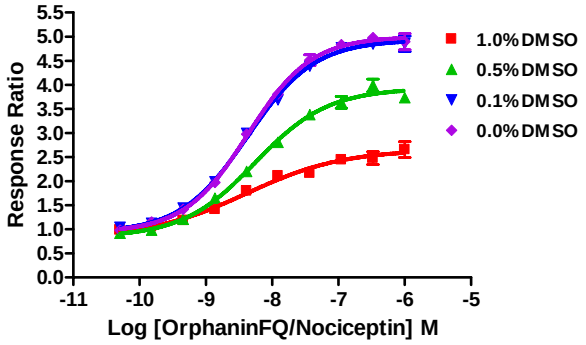
Figure 8 – GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to Orphanin FQ with different FBS concentrations



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 cells (10,000 cells/well) were plated in a poly-D-lysine treated 384-well plate and incubated for 16-20 hours. Cells were then stimulated with a dilution series of Orphanin FQ (Sigma #O4011) for 5 hours. Cells were seeded and Orphanin FQ dilutions were performed in the indicated media and FBS concentrations. Cells were then loaded for 1.5 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each FBS concentration against the indicated concentrations of Orphanin FQ (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

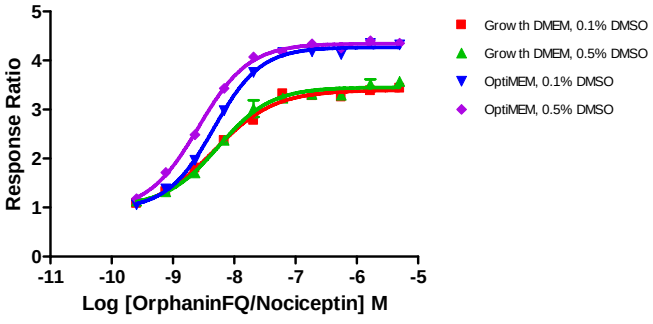
Figure 9 – GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to Orphanin FQ/Nociceptin with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 cells (10,000 cells/well) were plated in a tissue culture treated 384-well plate and incubated for 16-20 hours. Cells were stimulated with a dilution series of Orphanin FQ/Nociceptin (Sigma #O4011) for 5 hours. DMSO was added to the cells at concentrations from 0% to 1%. Cells were then loaded for 1.0 hour with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each DMSO concentration against the indicated concentrations of Orphanin FQ/Nociceptin (n=8 for each data point).

Assay Performance with Variable Media Formulation and % DMSO

Figure 10 – GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 dose response to OrphaninFQ with different media formulations



GeneBLAzer® OPRL1-Gqi5-NFAT-*bla* FreeStyle™ 293 cells (7,500 cells/well) were plated in a poly-D-lysine treated 384-well plate with the indicated media (growth DMEM or OptiMEM with 10% dFBS) and incubated for 16-20 hours. Cells were stimulated with a dilution series of OrphaninFQ (Sigma O4011) for 4 hours. Cells were seeded and OrphaninFQ dilutions were performed in the indicated media and percent DMSO. Cells were then loaded for 1.0 hour with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard florescence plate reader and the Response Ratios plotted for each FBS concentration against the concentrations of OrphaninFQ (n=8 for each data point).

References

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