GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1533

Cell Line Descriptions

The GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells and the GeneBLAzer® OPRK1 –Gqo5 CHO-K1 DA cells contain the Opioid Receptor kappa 1 (OPRK1) receptor (Accession # NM_000912) stably integrated in the CellSensor™ Gqo5-NFAT-*bla* CHO-K1 cell line (Cat No.K1536) contains the chimeric G protein and the beta-lactamase (*bla*) reporter gene under the control of the Nuclear Factor of Activated T Cells (NFAT) Response Element.

The GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of U50488 Figure 1). In addition, GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 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

Opioids have been known analgesics and anti-diuretics for over 5000 years. However, it wasn't until 1954 that Beckett and Casy discovered the opioid receptors in the body (1). Experiments on opioid receptors in the 60's and 70's led scientists to believe that there is more than one type of opioid receptor in the body (2, 3). The receptors were classified as μ (mu), κ (kappa) and δ (delta) after the drugs (morphine, ketocyclazocine and deferense, respectively) that were used to differentiate these receptors (4). The cloning of these opioid receptor sub-types also gave rise to the discovery of the Opioid Receptor Like 1 (OPRL1) receptor (5).

Opioid Receptor Kappa 1 (OPRK1) is known to regulate pain and pain perception. The OPRK1 receptors are involved in the production of urine by the kidneys leading to the increased occurrence of urination. OPRK1 is also found to regulate feeding, inhibit neurotransmitter release, and regulate temperature and modulate cardio respiratory function. (6, 7). The OPRK1 agonists also cause dysphoria which can lead to addiction as users seek the high.

OPRK1 receptors are found mostly in the brain with the highest levels in the cerebral cortex, hypothalamus and nucleus accumbens (8, 9). The receptors are also found in the gastrointestinal tract, immune cells and various peripheral tissues.

In the OPRK1-Gqi5-NFAT-bla CHO-K1 cell line the beta-lactamase gene was linked to an NFAT response element. When intracellular calcium levels rise the NFAT is activated inducing transcription of the beta-lactamase gene.

Validation Summary

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

1. **Primary agonist dose response under optimized conditions(n=3)**

	DA Cells	Live Cells
EC_{50}	13.7 nM	4.1 nM
Z'-Factor	0.81	0.71
Recommended cell no.		= 10K cells/well
Recommended [DMSO]		= up to 1%
Recommended Stim. Time		= 5 hours
Max. [Stimulation]		= 50 µM

2. **Alternate agonist dose response**

GR89696 (EC_{50})	= 0.849 nM
U69593 (EC_{50})	= 102 nM

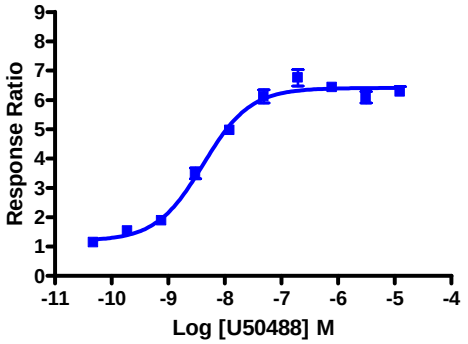
3. **Antagonist dose response**
   See antagonist dose response section

Assay Testing Summary

4. **Assay performance with variable cell number**
5. **Assay performance with variable stimulation time**
6. **Assay performance with variable substrate loading time**
7. **Assay performance with variable DMSO concentration**
8. **Assay performance in 2nd messenger assay.**

Primary Agonist Dose Response

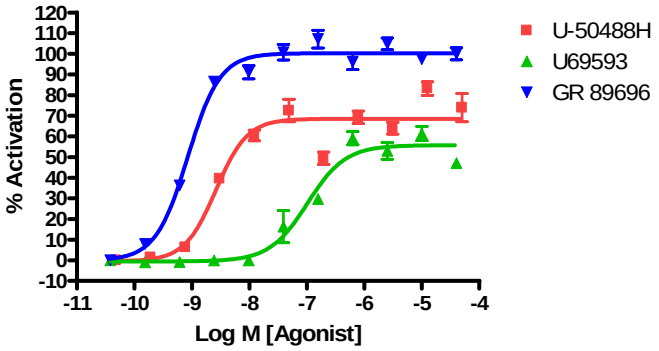
Figure 1 — GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells dose response to U50488 under optimized conditions



GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed from and assay media added. Cells were stimulated with a dilution series of U-50488H (Sigma D8040) in the presence of 0.5% DMSO for 5 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 U-50488H (n=16 for each data point).

Alternate Agonist Dose Response

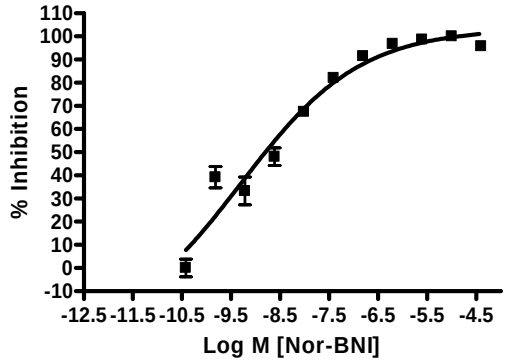
Figure 2 —U-50488H, GR 89696 and U69593 Dose Response



GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Growth medium was removed and replenished with assay medium. Cells were stimulated with dilution series of U-50488H (Sigma D8040), GR 89696 (Sigma G133) and U69593 (Sigma U103) in the presence of 0.5% DMSO for 5 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 florescence plate reader and the % Activation is shown plotted against the concentrations of U-50488H and Adenosine (n=8 for each data point). The cell lines show the correct rank order potency for these compounds.

Antagonist Dose Response

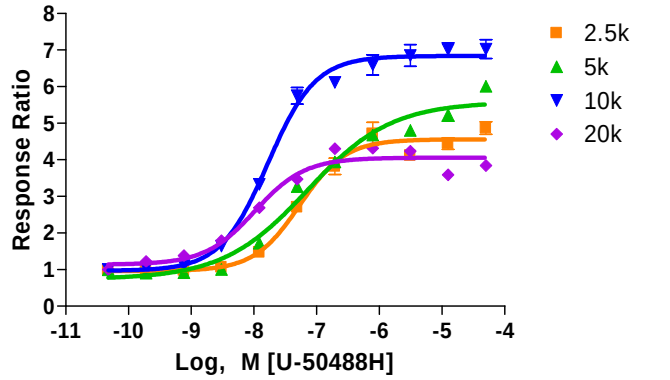
Figure 3 — Nor-BNI and Naltrexone dose response



GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells were plated 16-20 hours prior to assay at 10,000 cells per well in a 384-well format. A dilution series of Nor BNI (Sigma N1771) in the presence of 0.25% DMSO. Cells were incubated at 37ºC & 5% CO_2 for 30 min. U-50488H (Sigma D8040) was added to the plate at the EC_{80} concentration of 40 nM along with 0.25% DMSO (0.5% Final concentration). Cells were incubated for 5 hours and loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and % Inhibition plotted for each replicate against the concentrations of the antagonist. This data shows the proper functioning of the assay in antagonist mode. (N=16 for each data point).

Assay Performance with Variable Cell Number

Figure 4 – U-50488H dose response with 2.5, 5, 10, and 20K cells / well



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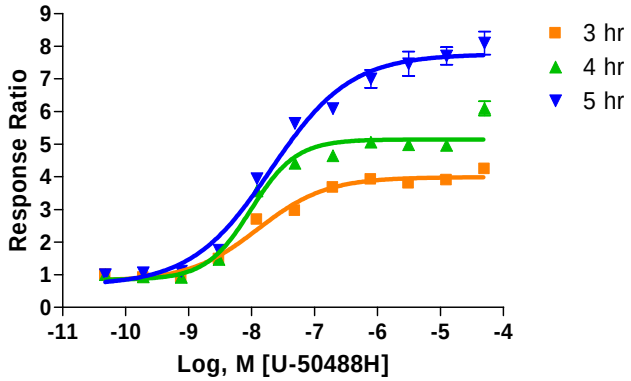
GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells were plated at 2,500 5,000 10,000 or 20,000 cells/well in a 384-well format and incubated for 16-20 hours. Growth medium was removed and replenished with assay medium. Cells were stimulated with a dilution series of U-50488H (Sigma D8040) in the presence of 0.5% DMSO for 5 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 and the Response Ratios plotted for each cell number against the concentrations of U-50488H (n=8 for each data point).

Assay Performance with Variable Stimulation Time

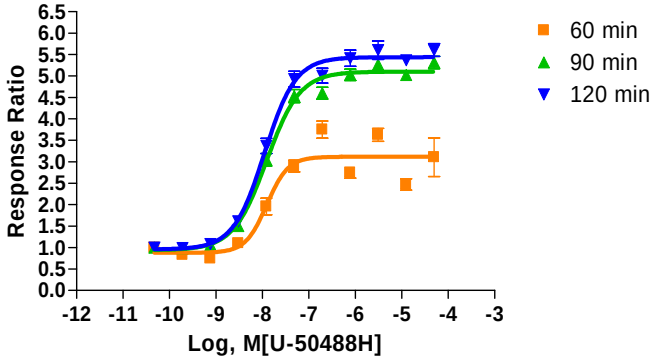
Figure 5 – U-50488H dose response with 3, 4 and 5 hr stimulation times



GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Growth medium was removed and replenished with assay medium. Cells were stimulated with a dilution series of U-50488H (Sigma D8040) for 3, 4, or 5 hrs in the presence of 0.5% DMSO. Cells were then loaded for 2 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 concentrations of U-50488H (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

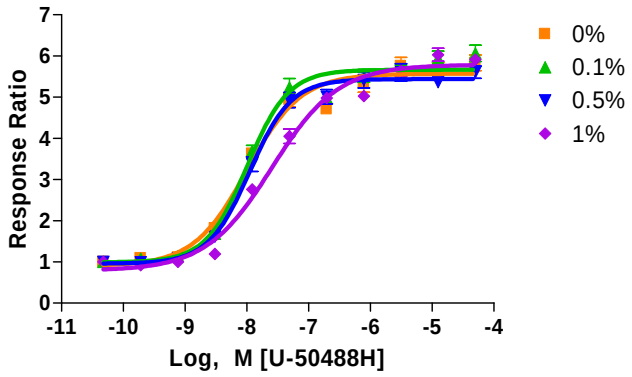
Figure 6 – U-50488H dose response with 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Growth medium was removed and replenished with assay medium. Cells were stimulated with a dilution series of U-50488H (Sigma D8040) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for either 1, 1.5 or 2 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 concentrations of U-50488H (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

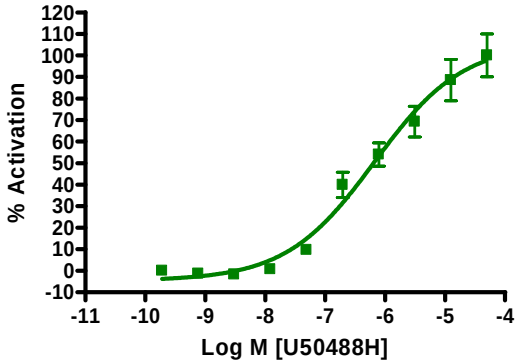
Figure 7 – U-50488H dose response with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Growth medium was removed and replenished with assay medium. Cells were stimulated with a dilution series of U-50488H (Sigma D8040) for 5 hours. DMSO was added to the cells at concentrations from 0% to 1%. Cells were then loaded for 2 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 DMSO concentration against the concentrations of U-50488H (n=8 for each data point).

2nd Messenger Dose Response

Figure 8 —2nd messenger dose response to U-50488H under optimized conditions.



GeneBLAzer® OPRK1-Gqo5-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to U50488H.

References

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