GeneBLAzer® MCHR1-Gqi5 CHO-K1 DA Assay Kit

GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1355 and K1730

Cell Line Descriptions

GeneBLAzer® MCHR1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells contain the human Melanin-concentrating hormone receptor (MCHR1), (Accession # NM_005297) stably integrated into the GeneBLAzer® Gqi5 NFAT-*bla* CHO-K1 cell line. GeneBLAzer® Gqi5-NFAT-*bla* CHO-K1 cells (#K1725) contain a beta-lactamase (*bla*) reporter gene under control of the nuclear factor of activated T-cells (NFAT) response element, and also stably express the chimeric protein, Gqi5. Division Arrested (DA) cells are available as an Assay Kit, which includes cells and sufficient substrate to analyze 1 x 384-well plate.

DA cells are irreversibly division arrested using a low-dose treatment of Mitomycin-C, and have no apparent toxicity or change in cellular signal transduction. Both GeneBLAzer® MCHR1-Gqi5 CHO-K1 DA cells and GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of melanin concentrating hormone (MCH), (Figure 1). In addition, GeneBLAzer® MCHR1-Gqi5-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 (data available upon request). Additional testing data using alternate stimuli are also available.

Target Description

MCHR1 was originally identified as an orphan G protein-coupled receptor named somatostatin-like receptor (SLC-1) due to its 40% amino acid homology to five known somatostatin receptors, all of which are Gαi-linked (1). Melanin concentrating hormone (MCH) was identified as an agonist for MCHR1 upon screening of HEK293 cells expressing MCHR1 with a library of bioactive neuropeptides (2). Subsequent studies have identified a second family member, MCHR2, with 32% sequence homology to MCHR1 (3). Like MCHR1, the MCHR2 receptor responds to the agonist effects of MCH (3, 4).

MCHR1 expression is found in several regions of the brain including: olfactory tubercle, cerebral cortex, hippocampus, substantia nigra, amygala, medial nucleus accumbens, locus ceruleus, thalamus, and hypothalamus. Particularly high levels of MCHR1 mRNA are found in regions of the hypothalamus that regulate feeding behavior (1, 2). The agonist MCH has been shown to play a role in regulating body weight with increased levels in genetically obese and fasting mice while introducing MCH into rats leads to increase feeding (5, 6). Knocking out MCHR1 in mice leads to lean phenotype (7), while transgenic mice that overexpress MCH are obese (8).

Validation Summary

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

1. **MCH agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	19 nM	11 nM
Z'-factor	0.89	0.87

Optimum cell no.	= 5K cells/well
Optimum [DMSO]	= up to 1.0%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 1µM

2. **Alternate agonist dose response**

MCH, salmon EC_{50}	= 52 nM
[Phe^{13},Tyr^{19}]-MCH EC_{50}	= 41 nM

3. **Antagonist dose response**

ATC0175 IC_{50}	= 1.4 nM

4. **Agonist dose response using Fluo-4NW**

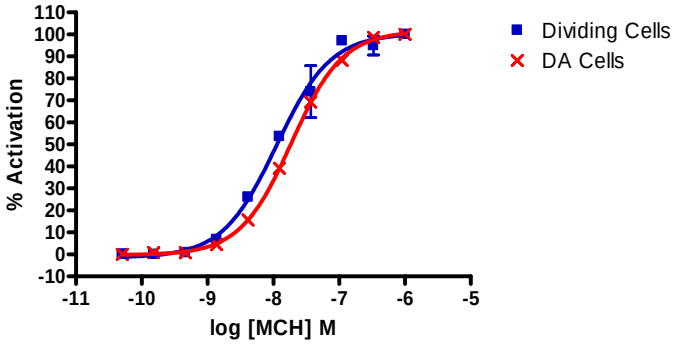
MCH EC_{50}	= 4.4 nM

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 DMSO concentration**

Primary Agonist Dose Response

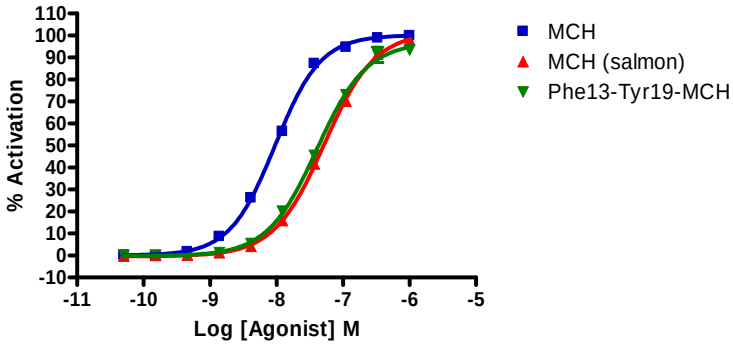
Figure 1 — GeneBLAzer® MCHR1-Gqi5 CHO-K1 DA and GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 dose response to MCH under optimized conditions



GeneBLAzer® MCHR1-Gqi5 CHO-K1 DA cells and GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of MCH 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 the % Activation plotted for each replicate against the indicated concentrations of MCH (n=6 for each data point).

Alternate Agonist Dose Response

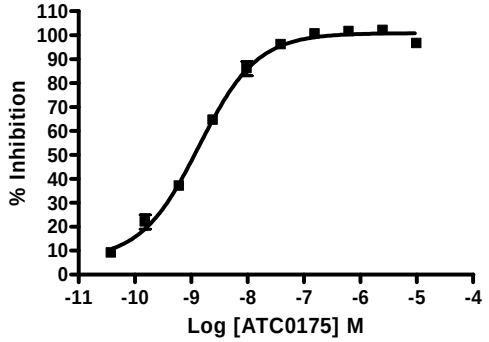
Figure 2 — GeneBLAzer® MCHR1-Gqi5-NFAT-bla CHO-K1 dose response to MCH, MCH(salmon) and [Phe^{13},Tyr^{19}]-MCH



GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells were plated at 5,000 cells/well in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of MCH (Sigma #M4542), salmon MCH (Sigma #M8441), and [Phe^{13},Tyr^{19}]-MCH (Phoenix Pharmaceuticals #070-45) 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 % Activation of each agonist plotted against the indicated concentrations of the agonists (n=8 for each data point).

Antagonist Dose Response

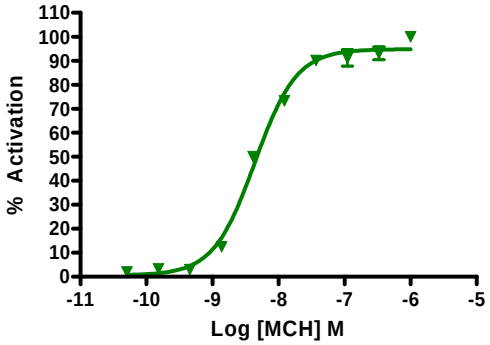
Figure 3 — GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 dose response to ATC0175



GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells (5,000 cells/well) plated in a 384-well format and incubated for 16-20 hours. Cells were then incubated with a dilution series ATC0175 or 30 min. at 37°C followed by a 5 hour incubation with an EC_{80} concentration of MCH (Sigma #M4542) in 0.1% DMSO. Fluorescence emission values at 460 nM and 530 nm were obtained and the % Inhibition plotted against the indicated concentrations of ATC0175 (n=2 for each data point).

Agonist 2nd Messenger Response

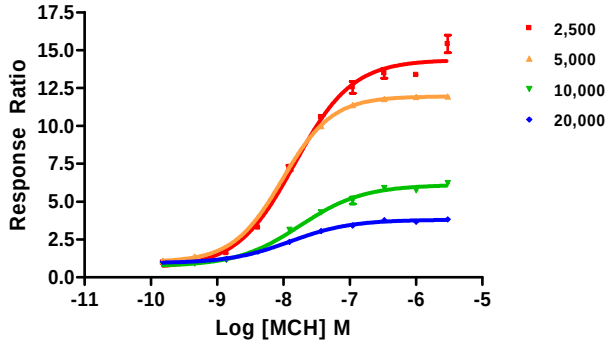
Figure 4 — GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 dose response to MCH using Fluo-4NW



GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells (5,000 cells/well) plated in a 384-well format and incubated for 16-20 hours. Cells were then incubated with Fluo-4NW for 30 min. at 37°C, followed by 30 min. at room temperature. Cells were then stimulated with a dilution series of MCH (Sigma #M4542) in the presence of 0.5% DMSO. Fluorescence emission values at 516 nm were obtained and % Activation plotted against the indicated concentrations of MCH (n=16 for each data point).

Assay Performance with Variable Cell Number

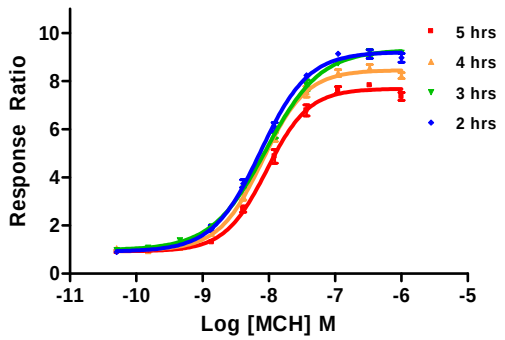
Figure 5 – GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 dose response to MCH with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® MCHR1-Gqi5-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. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of MCH (Sigma #M4542) 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 for each cell number plotted against the concentrations of MCH (n=8 for each data point).

Assay Performance with Variable Stimulation Time

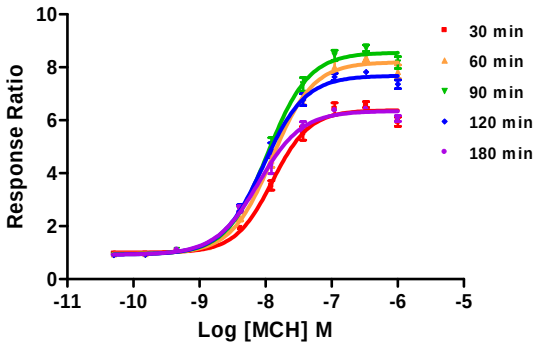
Figure 6 – GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 dose response to MCH with 2, 3, 4 and 5 hr stimulation times



GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of MCH (Sigma #M4542) for 2, 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 for each stimulation time plotted against the concentrations of MCH (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

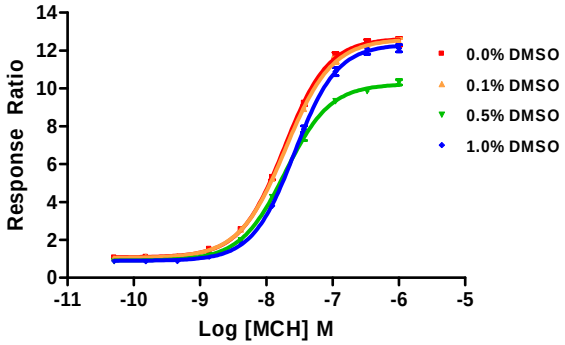
Figure 7 – GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 dose response to MCH with 0.5, 1, 1.5, 2, and 3 hr substrate loading times.



GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of MCH (Sigma #M4542) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for 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 for each substrate loading time plotted against the concentrations of MCH (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 dose response to MCH with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® MCHR1-Gqi5-NFAT-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of MCH (Sigma #M4542) 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 for each DMSO concentration plotted against the indicated concentrations of MCH (n=8 for each data point).

References

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