GeneBLAzer® MC3R CHO-K1 DA Assay Kit

GeneBLAzer® MC3R CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1423 and K1744

Cell Line Descriptions

GeneBLAzer® MC3R CHO-K1 DA (Division Arrested) cells and GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells contain the human Melanocortin subtype 3 receptor (MC3R), (Accession # NM_019888.2) stably integrated into the CellSensor® CRE-*bla* CHO-K1 cell line. CellSensor® CRE-*bla* CHO-K1 cells (Cat. no. K1535) contain a beta-lactamase (*bla*) reporter gene under control of the Cyclic AMP Response Element (CRE). 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® MC3R CHO-K1 DA cells and GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of NDP-α-MSH (Figure 1). In addition, GeneBLAzer® MC3R-CRE-*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

The melanocortin system consists of the five melanocortin G-Protein-coupled receptors (MC1-5R), the melanocortin peptides α-, β-, and γ-melanocyte-stimulating hormone (α-, β- and γ-MSH), the adrenocorticotropic hormone (ACTH), and two inverse agonists, agouti and agouti-related protein (AGRP) (1). α-, β- and γ-MSH are posttranslational products of the proopiomelanocortin (POMC) prohormone. The POMC gene is expressed primarily in the central nervous system (CNS), but is also expressed by cutaneous keratinocytes and melanocytes, and in some peripheral tissues including skin. POMC is differentially processed in a tissue specific manner. The agouti gene is expressed primarily in follicular epithelial cells, and the AGRP gene is expressed in the arcuate nucleus of the hypothalamus and in the adrenal gland (2-3). The physiology of the melanocortin receptors is simplified by the largely discreet distribution of the five receptors. The MC_1 receptor is made primarily in melanocytes (4).

The melanocortin-3 receptor (MC3R) is primarily expressed in the hypothalamus (5-6), and plays an important role in the regulation of energy homeostasis (7-8). MC3R recognizes all of the melanocortin peptides, and notably binds γ-MSH with greater affinity than the other melanocortin receptors (1). MC3R-deficient (*MC3R*-/-) mice demonstrate increased fat mass, higher feeding efficiency, and reduced lean body mass (9). To date only one mutation of MC3R has been identified to be associated with human obesity (10). Functional analysis of this mutation indicated a complete loss of agonist-mediated receptor activation (11).

Validation Summary

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

1. **NDP-α-MSH agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	29 pM	19 pM
Z'-factor	0.81	0.71

Recommended cell no. = 10K cells/well
Recommended [DMSO] = up to 1%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = 100 nM

2. **Alternate agonist dose response**

MTII EC_{50} = 146 pM

3. **Antagonist dose response**

SHU9119 IC_{50} = 36 nM

4. **Agonist 2nd messenger dose response**

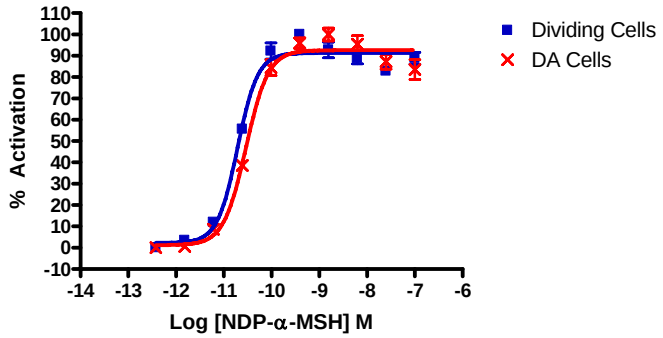
NDP-α-MSH EC_{50} = 1.3 nM

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**
9. **Assay Performance with different lot #s of FBS**

Primary Agonist Dose Response

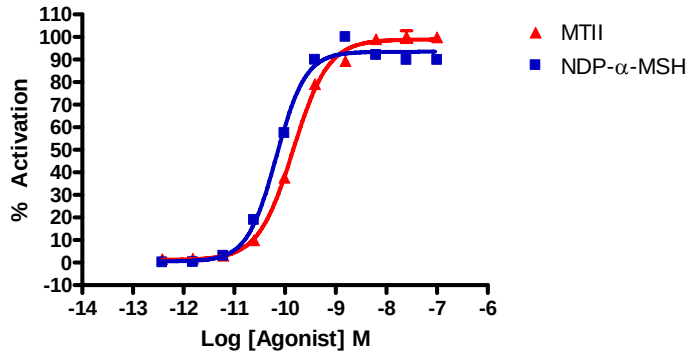
Figure 1 — GeneBLAzer® MC3R CHO-K1 DA and GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH under optimized conditions



GeneBLAzer® MC3R CHO-K1 DA cells and GeneBLAzer® MC3R-CRE-*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 NDP-α-MSH 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 NDP-α-MSH (n=6 for each data point).

Alternate Agonist Dose Response

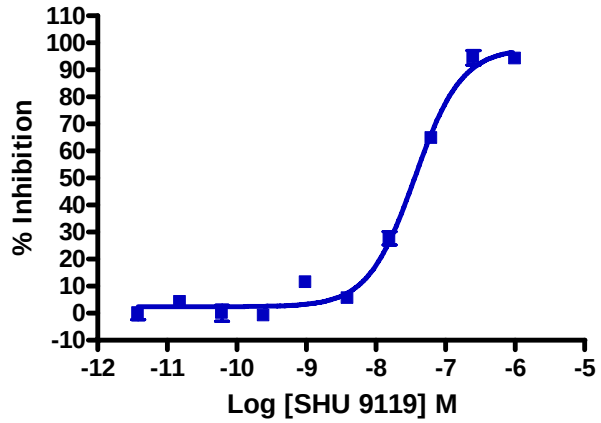
Figure 2 — GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to MTII and NDP-α–MSH.



GeneBLAzer® MC1R-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format prior to stimulation with Melanotan II (Phoenix Pharmaceuticals #043-23), or NDP-α-MSH (Phoenix Pharmaceuticals #043-06) over the indicated concentration range in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for 2 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Activation plotted against the indicated concentrations of agonist (n=8 for each data point). The data shows the correct rank order potency.

Antagonist Dose Response

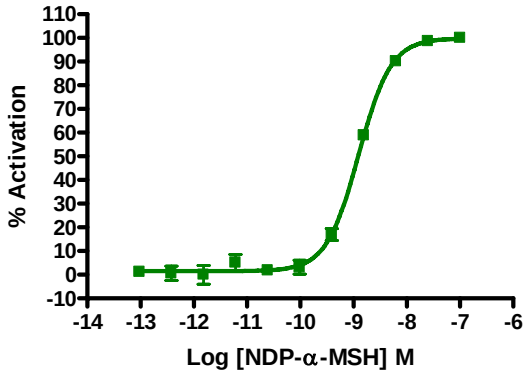
Figure 3 — GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to SHU-9119



GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells were plated the day before the assay at 10,000 cells/well in a 384-well format. On the day of the assay, cells were exposed to SHU9119 (Sigma #M4603) for 30 min. and then stimulated with an EC80 concentration of NDP-α-MSH (Phoenix Pharmaceuticals #043-06) 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of NDP-α-MSH (n=16 for each data point).

Agonist 2nd Messenger Response

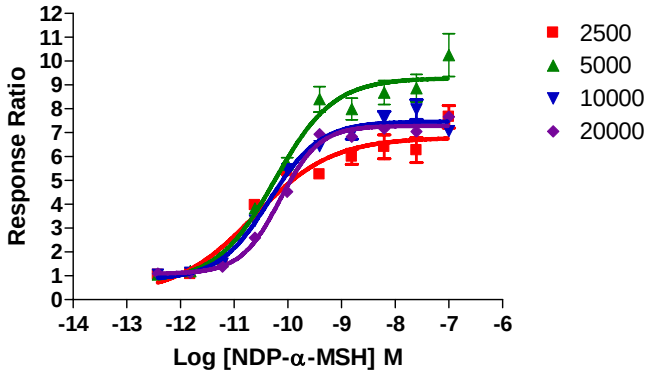
Figure 4— GeneBLAzer® MC3R-CRE-*bla* CHO-k1 2nd messenger dose response to NDP-α-MSH under optimized conditions



GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells were tested for a response to NDP-α-MSH with a TR-FRET cAMP assay

Assay Performance with Variable Cell Number

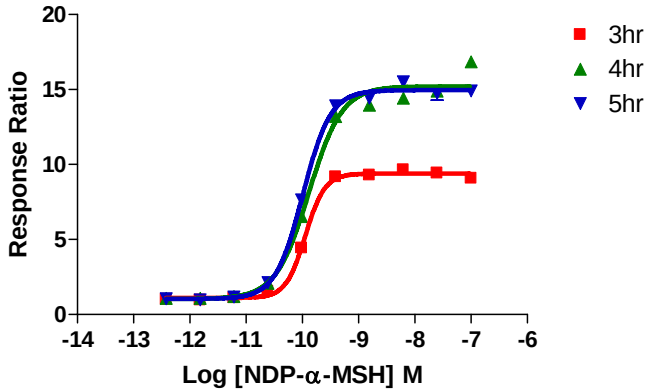
Figure5 — GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to NDP- α-MSH with 2.5, 5, 10 and 20K cells/well



GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells were plated the day before the assay at 2,500 5,000 10,000 or 20,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with NDP-α-MSH (Phoenix Pharmaceuticals #043-06) 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 for the various cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of NDP-α-MSH (n=8 for each data point).

Assay Performance with Variable Stimulation Time

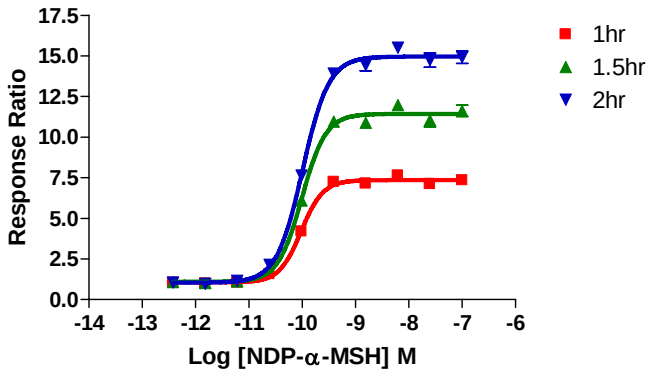
Figure 6 – GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH with 3, 4 and 5 hour stimulation times



GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. NDP-α-MSH (Phoenix Pharmaceuticals #043-06) was then added to the plate over the indicated concentration range for 3, 4, or 5 hrs in 0.5% DMSO. The 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 fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of NDP-α-MSH(n=16 for each data point).

Assay Performance with Variable Substrate Loading Time

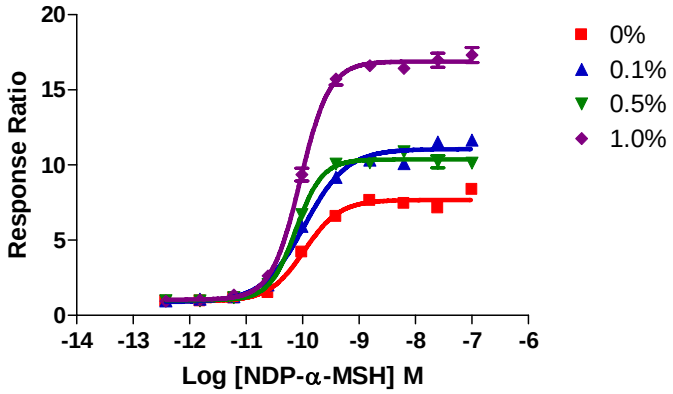
Figure 7 — GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH with 1, 1.5 and 2 hour substrate loading times



GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells were plated the day before the assay at 10,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with NDP-α-MSH (Phoenix Pharmaceuticals #043-06) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1, 1.5 or 2 hours. Fluorescence emission values at 460 nm and 530 nm for the various substrate loading times were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the indicated concentrations of NDP-α-MSH (n=16 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH with 0, 0.25, 0.5 and 1% DMSO



GeneBLAzer® MC3R-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. NDP-α-MSH (Phoenix Pharmaceuticals #043-06) was then added to the plate over the indicated concentration range in the presence of 0%, 0.1%, 0.5%, or 1% DMSO. Plates were stimulated for 5 hrs 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 the Response Ratios for each DMSO concentration were plotted against the indicated concentrations of α-MSH (n=8 for each data point).

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