GeneBLAzer® MC5R CHO-K1 DA Assay Kit

GeneBLAzer® MC5R CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1375 and K1740

Cell Line Descriptions

GeneBLAzer® MC5R CHO-K1 DA (Division Arrested) cells and GeneBLAzer® MC5R-CRE-*bla* CHO-K1 cells contain the human Melanocortin subtype 5 receptor (MC5R), (Accession # NM_005913) stably integrated into the CellSensor® CRE-*bla* CHO-K1 cell line. CellSensor® CRE-*bla* CHO-K1 cells (Cat. no. K1129) contain a beta-lactamase (*bla*) reporter gene under control of the Cyclic AMP Response Element (CRE). Division Arrested (DA) cells are available in 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® MC5R CHO-K1 DA cells and GeneBLAzer® MC5R-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of NDP-α-MSH, (Figure 1). In addition, GeneBLAzer® MC5R-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). The α- , β- and γ-MSH as well as the opioid peptide β-endorphin, are posttranslational products of the proopiomelanocortin (POMC) pro-hormone. 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 (1-2).

MC5 receptors are expressed in skin, muscle, lung, liver, spleen, and adrenal tissue (3, 4). MC5R is Gs coupled (4) and binds all of the melanocortin peptides and ACTH (1). A study by Zhang *et al* (5) suggests that MC5R is involved in fat storage in the sebocyte cells of the skin. Another study in mice links MC5R to aggressive behavior. Mice deficient in MC5R acted less aggressively when treated with alpha-MSH (6).

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}	33 pM	53 pM
Z'-factor	0.80	0.71

Recommended cell no.	= 10K cells/well
Recommended [DMSO]	= up to 1%
Recommended Stim. Time	= 4 hours
Max. [Stimulation]	= 10 nM

2. **Alternate agonist dose response**

MT II	EC_{50}	= 200 pM
HS024	EC_{50}	= 26 pM
SHU9119	EC_{50}	= 7.5 pM

3. **Antagonist Dose Response**
   There were no commercial sources of antagonist available for testing at the time of publication of this document.

4. **Agonist 2nd Messenger Dose Response**
   NDP-α-MSH EC_{50} = 353 pM

Assay Testing Summary

5. **Assay performance with variable cell number**

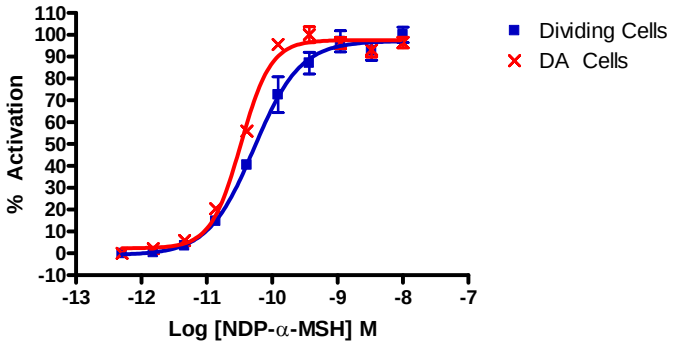
6. **Assay performance with variable stimulation time**

7. **Assay performance with variable substrate loading time**

8. **Assay performance with variable DMSO concentration**

Primary Agonist Dose Response

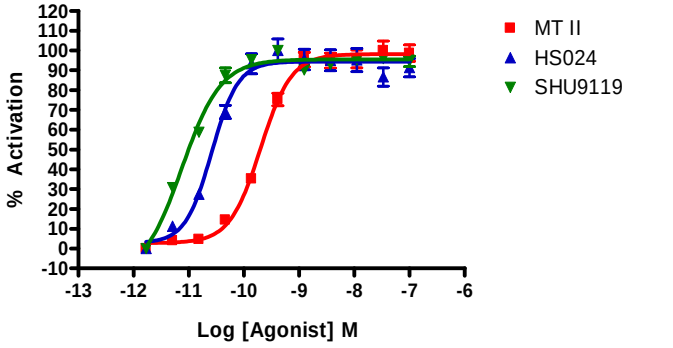
Figure 1 — GeneBLAzer® MC5R CHO-K1 DA and MC5R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH under optimized conditions



GeneBLAzer® MC5R CHO-K1 DA cells and GeneBLAzer® MC5R-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® MC5R-CRE-*bla* CHO-K1 dose response to various agonists under optimized conditions



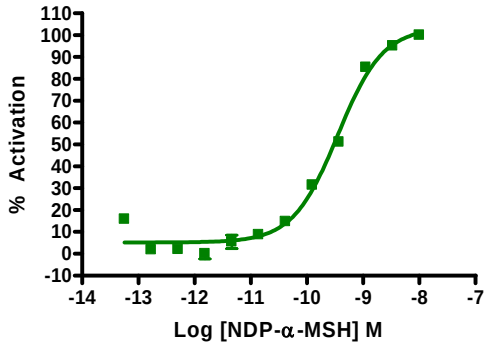
GeneBLAzer® MC5R-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format. On the day of the assay, cells were stimulated with MT II (Phoenix pharmaceuticals #043-23), HS024 (Phoenix Pharmaceuticals #043-27), or SHU 9119 (Sigma #M4603) in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate or 2 hours. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Activaton plotted against the indicated concentrations of the agonists (n=16 for each data point). The data shows the correct rank order potency of this agonist relative to α-MSH.

Antagonist Dose Response

There were no commercial sources of antagonist available for testing at the time of publication of this document.

Agonist 2nd Messenger Response

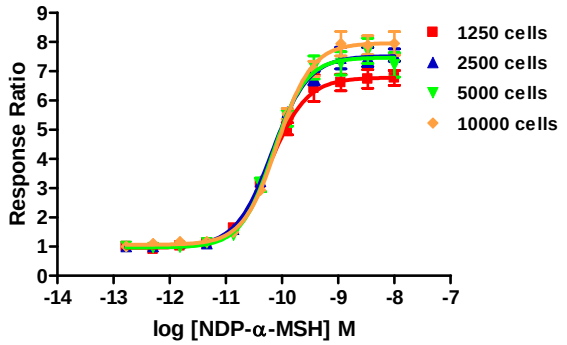
Figure 3— GeneBLAzer® MC5R-CRE-*bla* CHO-k1 2nd messenger dose response to NDP-α-MSH under optimized conditions



GeneBLAzer® MC5R-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

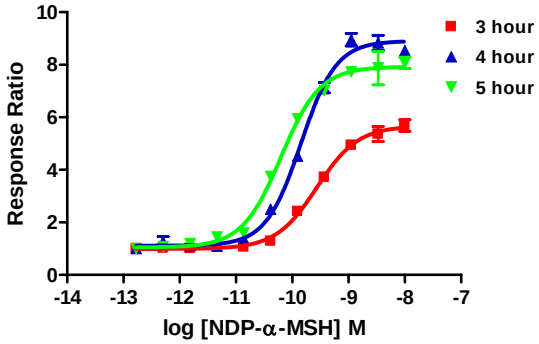
Figure4— GeneBLAzer® MC5R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH with 1.25, 2.5, 5 and 10K cells / well



GeneBLAzer® MC5R-CRE-*bla* CHO-K1 cells were plated the day before the assay at 1250 , 2500, 5,000 or 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 4 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

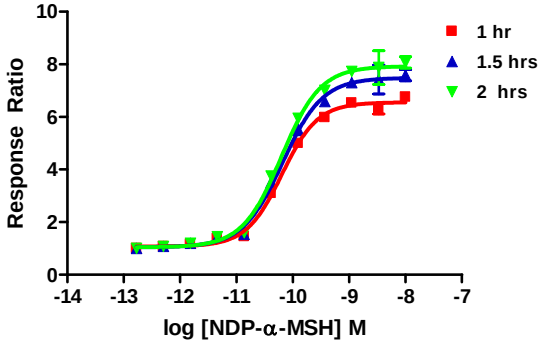
Figure 5 – GeneBLAzer® MC5R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH with 3, 4, and 5 hour stimulation times



GeneBLAzer® MC5R-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 for 3, 4 or 5 hrs in the presence of 0.5% DMSO and 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 (n=8 for each data point).

Assay Performance with Variable Substrate Loading Time

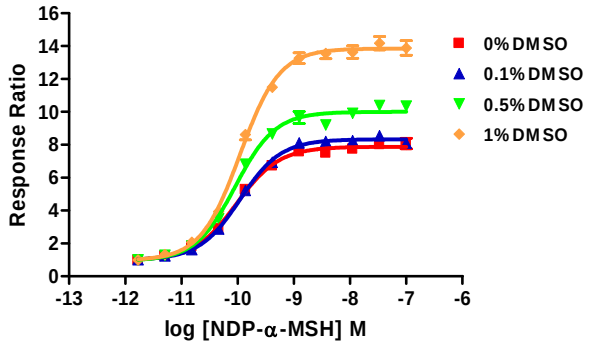
Figure 6 — GeneBLAzer® MC5R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH with 1, 1.5 and 2 hour substrate loading times



GeneBLAzer® MC5R-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 4 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=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 7 – GeneBLAzer® MC5R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH with 0, 0.1, 0.5 and 1% DMSO



Gene BLAzer® MC5R-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. DMSO was added to the assay at concentrations from 0% to 1%. NDP-α-MSH (Phoenix Pharmaceuticals #043-06) was then added to the plate for 4 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 florescence plate reader and the Response Ratios for each DMSO concentration were plotted against the indicated concentrations of NDP-α-MSH (n=8 for each data point).

References

1) Gantz, I., *et al* **(2003).** The melanocortin system, *Am. J. Phisiol. Endocrinol. Metab.*, **284**:E468-E474

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3) Gantz I, Shimoto Y, Konda Y, Miwa H, Dickinson CJ, Yamada T **(1994).** Molecular cloning, expression, and characterization of a fifth melanocortin receptor. *Biochem. Biophys. Res. Commun.* **200:**1214-1220

4) Labbe 0, Desarnaud F, Eggerickx D, Vassart G, Parmentier M **(1994).** Molecular cloning of a mouse melanocortin 5 receptor gene widely expressed in peripheral tissues. *Biochem*. **33:**4543-4549

5) Zhang, L., Li, W.H., Anthonavage, M., and Eisinger, M. **(2005).** Melanocortin-5 receptor: A marker of human sebocyte differentiation. *Peptides* **27:** 413-420.

6) Morgan, C. and Cone, R.D. **(2006).** Melanocortin-5 receptor deficiency in mice blocks a novel pathway influencing pheromone-induced aggression. *Behavioral Genetics* **36**: 291-300.