GeneBLAzer® MC2R-CRE-*bla*-CHOK1 DA Cells

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Catalog Numbers – K1597 and K1483

Cell Line Descriptions

GeneBLAzer® MC2R-CRE-*bla*-CHOK1 DA (Division Arrested) cells and GeneBLAzer® MC2R-CRE-*bla*-CHOK1 cells contain the human melanocortin-2 receptor (MC2R), (Accession # NM_000529.1) and the Melanocortin-2 receptor accessory protein (MRAP), (Accession #NM_178817.3) 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 cAMP Response Element (CRE).

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® MC2R-CRE-*bla*-CHOK1 DA cells and GeneBLAzer® MC2R-CRE-*bla*-CHOK1 cells are functionally validated for Z'-factor and EC_{50} concentrations of ACTH 1-24 (Figure 1). In addition, GeneBLAzer® MC2R-CRE-*bla*-CHOK1 cells have been tested for assay performance under variable conditions.

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	Dividing Cells
EC_{50}	7.4 pM	16 pM
Z'-factor	0.67	0.70

Recommended cell no.	= 10K cells/well
DMSO Tolerance	= up to 1.0%
Recommended Stim. Time	= 5 hours
Max. [Stimulation]	= 1 nM

2. **Alternate agonist dose response**

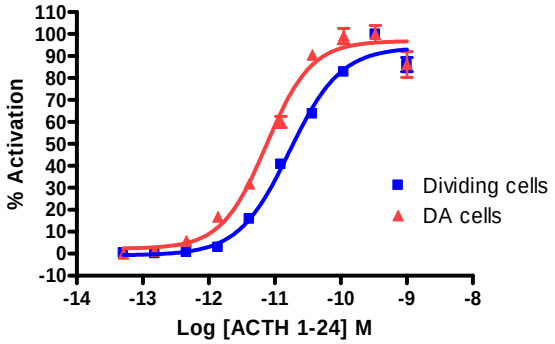
ACTH 1-39 (EC_{50})	= 12 pM
ACTH (EC_{50})	= 169 pM

Assay Testing Summary

3. **Assay performance with variable cell number**
4. **Assay performance with variable stimulation time**
5. **Assay performance with variable substrate loading time**
6. **Assay performance with variable DMSO concentration**

Primary Agonist Dose Response

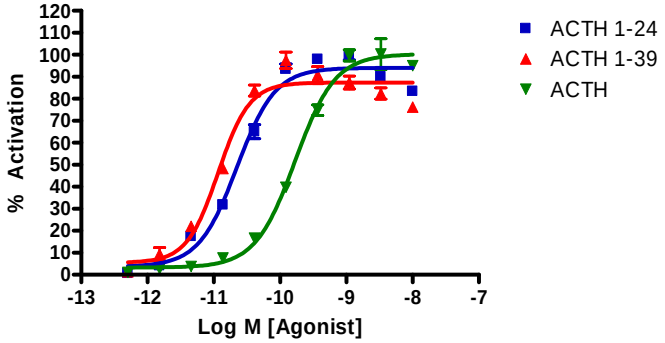
Figure 1 — GeneBLAzer® MC2R CHO-K1 DA and GeneBLAzer® MC2R-CRE-*bla* CHO-K1 dose response to NDP-α-MSH under optimized conditions



GeneBLAzer® MC2R-CRE-*bla* CHO-K1 and GeneBLAzer® MC2R CHO-K1 DA 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 adrenocorticotropin (ACTH) 1-24 (Sigma cat# A0298) 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 was plotted for each replicate against the concentrations of ACTH 1-24 (n=16 for each data point).

Alternate Agonist Dose Response

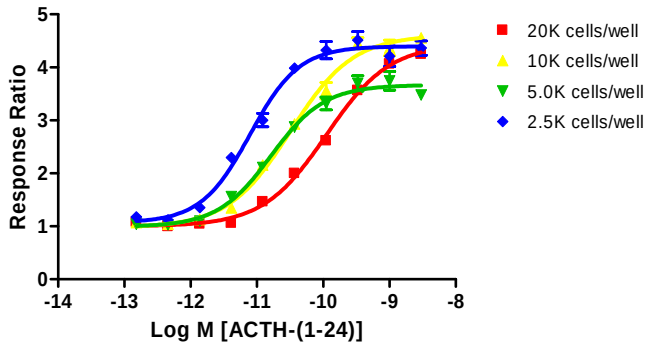
Figure 2 — GeneBLAzer® MC3R-CRE-*bla* CHO-K1 dose response to ACTH 1-24, ACTH 1-39 and ACTH



GeneBLAzer® MC2R-CRE-*bla* ChoK1 cells were plated at 10,000 cells/well in a 384-well plate and incubated for 16-20 hours. Cells were stimulated with a dilution series of adrenocorticotropin (ACTH) 1-24 (Sigma cat# A0298), adrenocorticotropin (ACTH) 1-39 (Bachem cat# H-1160), and adrenocorticotropin (ACTH) (Sigma cat# A0423) 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 is plotted for each cell number against the concentrations of the agonists (n=8 for each data point).

Assay Performance with Variable Cell Number

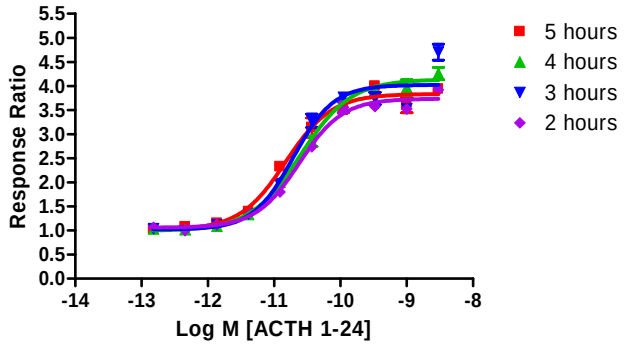
Figure 3 – GeneBLAzer® ACTH (1-24) dose response with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® MCR2-CRE-*bla* ChoK1 cells were plated at 2,500 5,000 10,000 or 20,000 cells/well in a black wall, clear bottom 384-well plate and incubated for 16-20 hours. Cells were stimulated with a dilution series of ACTH-(1-24) (Bachem cat# H1150) 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 ACTH-(1-24) (n=8 for each data point).

Assay Performance with Variable Stimulation Time

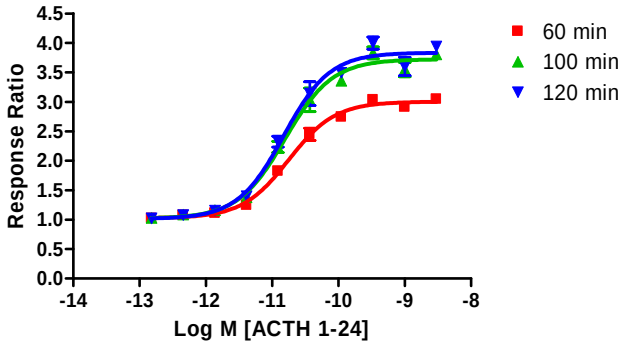
Figure 4 – GeneBLAzer® ACTH-(1-24) dose response with 2, 3, 4 and 5 hr stimulation times



GeneBLAzer® MC2R-CRE-*bla* ChoK1 cells were plated in a black walled, clear bottom 384-well plate at 5,000 cells/well, and incubated for 16-20 hours. Cells were stimulated with a dilution series of ACTH-(1-24) (Bachem cat# H1150) 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 plotted for each stimulation time against the concentrations of ACTH-(1-24) (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

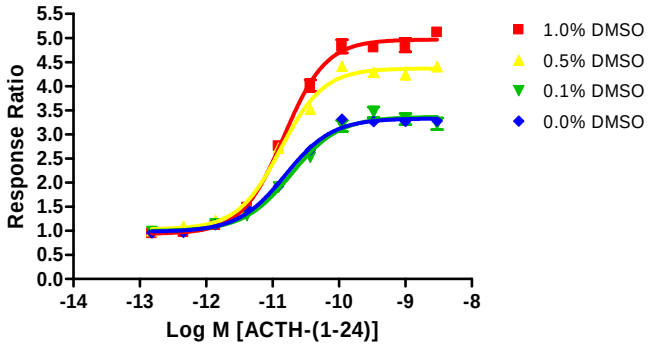
Figure 5 – GeneBLAzer® ACTH-(1-24) dose response with 1, 1.5, 2, and 2.5 hour substrate loading times.



GeneBLAzer® MC2R-CRE-*bla* ChoK1 cells (5,000 cells/well) were plated in a black walled, clear bottom 384-well plate and incubated for 16-20 hours. Cells were stimulated with a dilution series of ACTH-(1-24) (Bachem cat# H1150) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for either 60, 100, or 120 minutes 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 ACTH-(1-24) (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 6 – GeneBLAzer® ACTH-(1-24) dose response with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® MCR2-CRE-*bla* ChoK1 cells (5,000 cells/well) were plated in a black walled, clear bottom 384-well plate and incubated for 16-20 hours. DMSO was added to the cells at concentrations from 0% to 1%. Cells were stimulated with a dilution series of ACTH-(1-24) (Bachem cat# H1150) for 5 hours. 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 ACTH-(1-24) (n=8 for each data point.