GeneBLAzer® GPR54 CHO-K1 DA Assay Kit

GeneBLAzer® GPR54 NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1333 and K1720

Cell Line Descriptions

GeneBLAzer® GPR54 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 cells contain the human KiSS1 receptor (GPR54), (Accession # NM_032551) stably integrated into the CellSensor® NFAT-*bla* CHO-K1 cell line. CellSensor® NFAT-*bla* CHO-K1 cells (Cat. no. K1534) contain a beta-lactamase (*bla*) reporter gene under control of the Nuclear Factor of Activated T-cells (NFAT) response element. 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® GPR54 CHO-K1 DA cells and GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Metastin, (Figure 1). In addition, GeneBLAzer® GPR54-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

The KiSS-1/GPR54 system was initially identified for its involvement in tumor progression and metastasis (1, 2). Potent anti-metastasis actions of KiSS-1 peptide have been described in several tumors, such as papillary thyroid carcinoma, breast carcinoma, melanoma and bladder cancer (3, 4, 5, 6).

In 2003 the KiSS-1/GPR54 system was identified as a key player in the regulation of reproductive maturation. Loss-of function point mutations and deletions in the GPR54 coding sequence were shown to be associated with idiopathic hypogonadotropic hypogonadism (7, 8). Functional studies have more recently shown KiSS-1 signaling relevance in the onset of puberty in rodents and primates (9-12). The mechanistic affects of KiSS-1/GPR54 system stem from the direct stimulation of the hypothalamic gonadotropin-releasing hormone system (13).

The *KiSS-1* gene encodes a 145-amino-acid peptide that is proteolytically processed into the kisspeptins. The kisspeptins (also known as metastin) are peptide agonists of the GPR54 receptor. KiSS-1 is known to be highly expressed in the placenta and the brain (1, 14). GPR54 is widely expressed in human tissues including high expression in the placenta, brain, and pituitary (1, 14).

Validation Summary

Performance of this assay was validated under optimized conditions in 384-well format using LiveBLAzer™-FRET B/G Substrate.

1. **Metastin(45-54) agonist dose response under optimized conditions(n=4)**

	DA cells	Dividing Cells
EC_{50}	0.6 nM	0.6 nM
Z'-factor	0.93	0.78

Optimum cell no. = 10K cells/well
Optimum [DMSO] = up to 1%
Optimum Stim. time = 4 hours
Max. [Stimulation] = 1µM

2. **Alternate agonist dose response**

full length Metastin EC_{50} = 1 nM

3. **Antagonist dose response**
There were no sources of antagonist available for testing at the time of publication of this document

4. **Agonist dose response using Fluo-4NW**
Metastin(45-54) = 0.7 nM

Assay Testing Summary

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

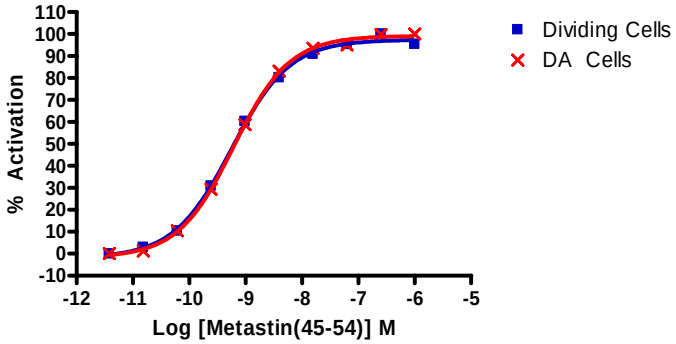
6. **Assay performance with variable stimulation time**

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

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

Primary Agonist Dose Response

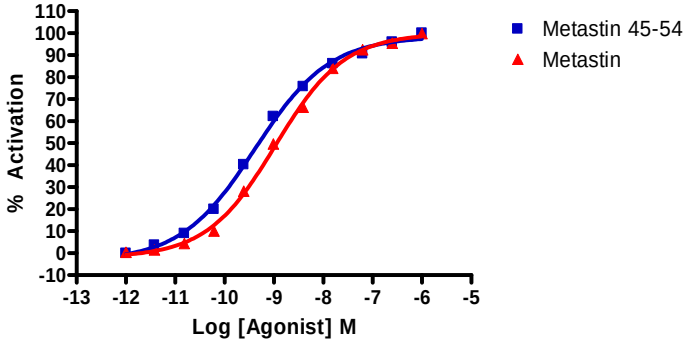
Figure 1 — GeneBLAzer® GPR54 CHO-K1 DA and GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 dose response to Metastin under optimized conditions



GeneBLAzer® GPR54 CHO-K1 DA cells and GeneBLAzer® GPR54-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 Metastin(45-54) 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 concentrations of Metastin(n=6 for each data point).

Alternate Agonist Dose Response

Figure 2 — GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 dose response to Metastin (45-54) and Metastin (full length)



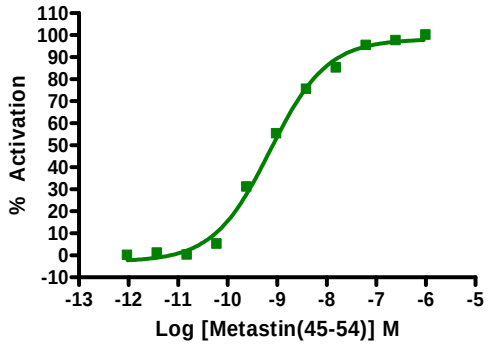
GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 cells (40,000 cells/well) were plated the day before the assay in a 96-well format. Cells were stimulated with either Metastin (45-54) (Calbiochem #445888), or full length Metastin (Calbiochem #445885) over the indicated concentration range in the presence of 0.5% 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 were obtained using a standard fluorescence plate reader and the % Activation is shown plotted against the indicated concentrations of the agonists (n= 16 for each data point).

Antagonist Dose Response

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

Agonist Dose Response using Fluo-4NW

Figure 3 — GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 dose response to Metastin using Fluo-4NW

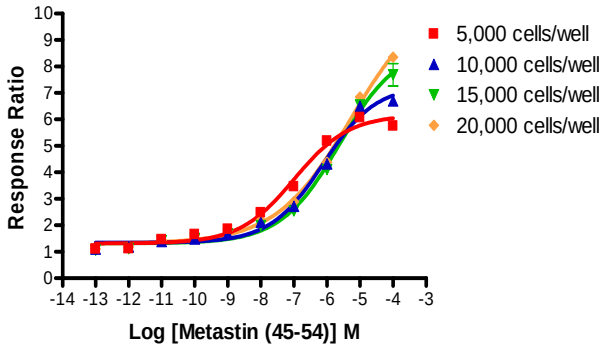


GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format. Cells were incubated with Fluo-4NW for 30 min. at 37°C, followed by 30 min. at room temperature. Cells were then stimulated with a dilution series of Metastin (Calbiochem #445885) in the presence of 0.5% DMSO. Fluorescence emission values at 516 nm were obtained and % Activation plotted against the indicated concentrations of agonist (n=16 for each data point).

Note: For all validation assays shown on the following page, the optimal protocol had not yet been developed leading to higher EC50 values.

Assay Performance with Variable Cell Number

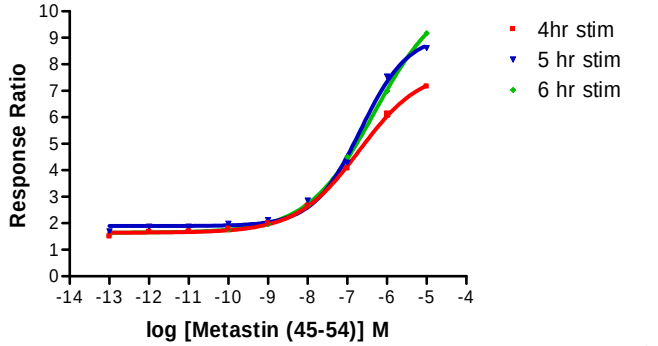
Figure 4— GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 dose response using 5, 10, 15 and 20K cells/ well



GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 cells were plated the day before the assay at 5000, 10,000 15,000 or 20,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with Metastin (45-54) (Calbiochem #445888) in the presence of 0.5% DMSO for 6 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 Metastin (45-54) (n=8 for each data point).

Assay performance with Variable Stimulation Time

Figure 5 – GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 dose response using 4, 5, and 6 hour stimulation times



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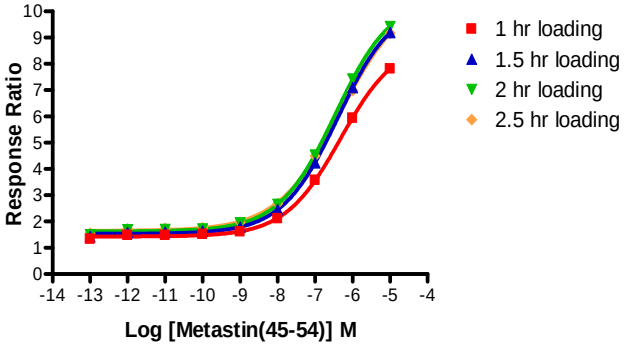
eneBLAzer® GPR54-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format. Metastin (45-54) (Calbiochem #445888) was then added to the plate over the indicated concentration range for 4, 5, or 6 hrs in 0.5% DMSO and then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratios for each substrate loading time were plotted against the indicated concentrations of Metastin (45-54) (n=16 for each data point)

Assay performance with Variable Substrate Loading Time

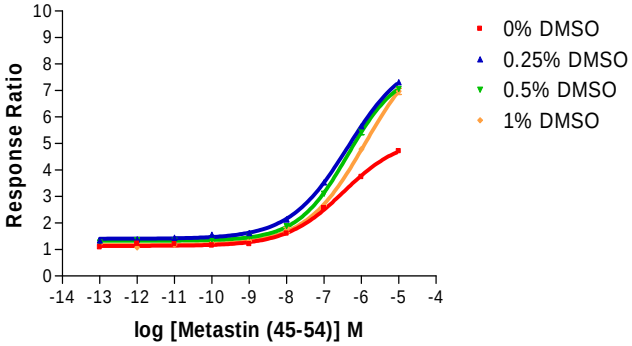
Figure 6 – GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 dose response using 1, 1.5, 2, and 2.5 hour substrate loading times



GeneBLAzer® GPR54-NFAT-*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 Metastin (45-54) (Calbiochem #445888) in the presence of 0.5% DMSO for 6 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate for either 1, 1.5, 2 or 2.5 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 Metastin (45-54) (n=16 for each data point).

Assay Performance with variable DMSO concentration

Figure 7 – Figure 5 – GeneBLAzer® GPR54-NFAT-*bla* CHO-K1 dose response using 0, 0.25, 0.5 and 1% DMSO



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eneBLAzer® GPR54-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format. Metastin (45-54) (Calbiochem #445888) was then added to the plate over the indicated concentration range. DMSO was added to the assay at concentrations from 0% to 1%. Cells were stimulated for 5 hrs with agonist 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 are for each DMSO concentration were plotted against the indicated concentrations of Metastin (45-54) (n=8 for each data point).

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