GeneBLAzer® GPR10 CHO-K1 DA Assay Kit

GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1359 and K1732

Cell Line Descriptions

GeneBLAzer® GPR10 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells contain the human Prolactin Releasing Peptide receptor (GPR10), (Accession #NM_004248) 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® GPR10 CHO-K1 DA cells and GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of PrRP20, (Figure 1). In addition, GeneBLAzer® GPR10-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

Prolactin is a multifunctional peptide produced in the pituitary that stimulates mammary tissue development and lactation. The Prolactin Releasing Peptide (PrRP) was first isolated from the bovine hypothalamus. Hypothalamic peptide hormones are responsible for the secretion of pituitary hormones from the anterior pituitary. The newly discovered bovine peptide was tested and found that it increased the secretion of prolactin from the anterior pituitary (1). It was also discovered that PrRP is found endogenously in two forms; a full length 31 amino acid peptide named PrRP31 and a trunctated 20 amino acid form named PrRP20 (2). Studies found PrRP in the hypothalamus, pituitary, medulla oblongata and various tissues through out the body (1).

The orphan receptor GPR10 (PrRPR) was identified as the receptor for PrRP (3). GPR10 is expressed in the Central Nervous System with the highest levels being found in the pituitary. The receptor is also found in the hypothalamus, thalamus, spinal cord and brainstem (2).

Two main areas of disease relevance involve the GPR10 pathway. The first is obesity, which was prompted by a study in rats which showed a reduction in appetite and food intake when treated with GPR10 agonist. This study in mice was unable to be repeated in humans (4-5); however, when studying GPR10's role in obesity a second area of disease relevance was discovered (blood pressure regulation). Two studies performed in the UK found two prevalent polymorphisms (G-62A (diastolic) & C914T (systolic/diastolic)), which resulted in people that were least likely to benefit from change in diet and exercise (6).

Validation Summary

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

1. **PrRP20 agonist dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	28 pM	45 pM
Z'-factor	0.88	0.86

Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 1%
Optimum Stim. Time	= 4-5 hours
Max. [Stimulation]	= 25nM

2. **Alternate agonist dose response**

PrRP31 EC_{50}	= 63pM

3. **Antagonist dose response**
   There are no known antagonists for the GPR10 receptor at the time of publication of this document.

4. **Agonist 2nd messenger response**

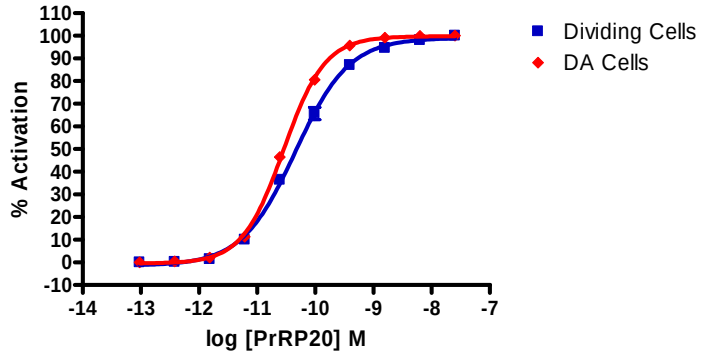
PrRP20 EC_{50}	= 2.5nM

Assay Testing Summary

5. **Assay performance using variable cell # / well**
6. **Assay performance using variable stimulation time**
7. **Assay performance using variable substrate loading times**
8. **Assay performance using variable DMSO concentration**
9. **Assay performance using Various Plating Times**
10. **Assay performance using dissociation methods**

Primary Agonist Dose Response

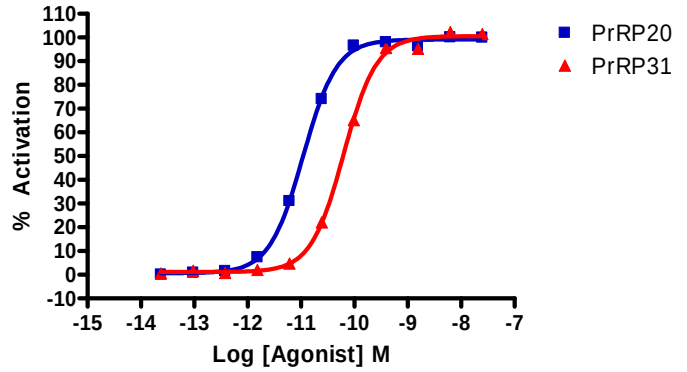
Figure 1 — GeneBLAzer® GPR10 CHO-K1 DA and GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response to PrRP20 under optimized conditions



GeneBLAzer® GPR10 CHO-K1 DA cells and GeneBLAzer® GPR10-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 PrRP20 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 plotted for each replicate against the concentrations of PrRP20 (n=6 for each data point).

Alternate Agonist Dose Response

Figure 2 — GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response to PrRP20 and PrRP31



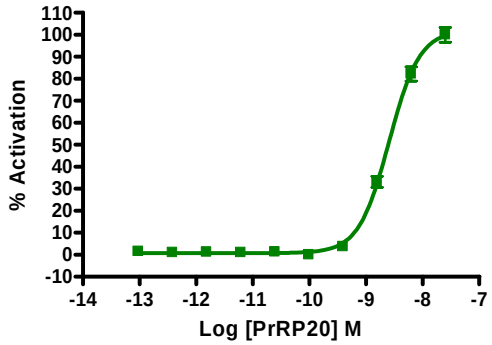
GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with a dilution series of either PrRP20 (Sigma P7107), or PrRP31 (Sigma P6982) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET 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 Emission Ratios plotted against the indicated of the agonists (n= 8 for each data point). The data shows the correct rank order potency for these agonists.

Antagonist Dose Response

There are no known antagonists for the GPR10 receptor at the time of publication of this document.

Agonist 2nd Messenger Response

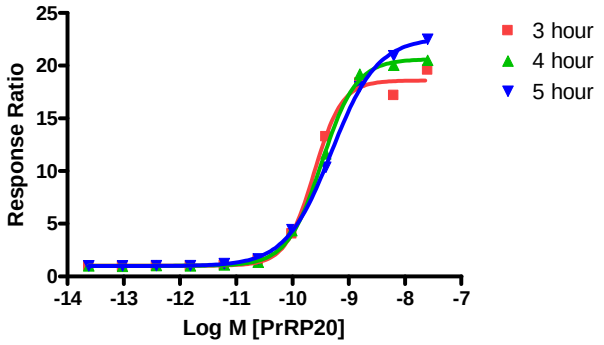
Figure 4— GeneBLAzer® GPR10-NFAT-*bla* CHO-k1 2nd messenger dose response to PrRP20 under optimized conditions



GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to PrRP20.

Assay performance with Variable Stimulation Time

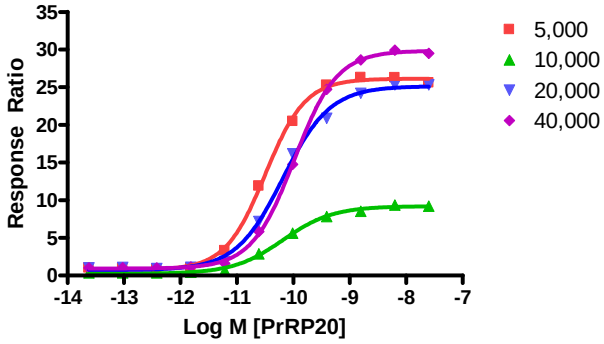
Figure 6 – GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response using 3, 4, and 5 hour stimulation times



GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format. Cells were stimulated with a dilution series of PrRP20 (Sigma P7107) for 3, 4, or 5 hrs in 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 fluorescence plate reader. Response Ratios are shown plotted against the concentrations of PrRP20 (n=8 for each data point).

Assay Performance with Variable Cell Number

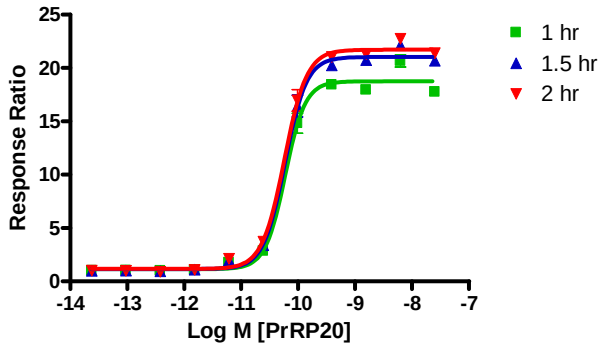
Figure 5— GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response using 5, 10, 20, and 40K cells/well



GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells were plated at 5000, 10,000, 20,000 or 40,000 cells/well in a 384-well format. Cells were stimulated with a dilution series of PrRP20 (Sigma P7107) 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 concentrations of PrRP20 (n=8 for each data point).

Assay performance with Variable Substrate Loading Time

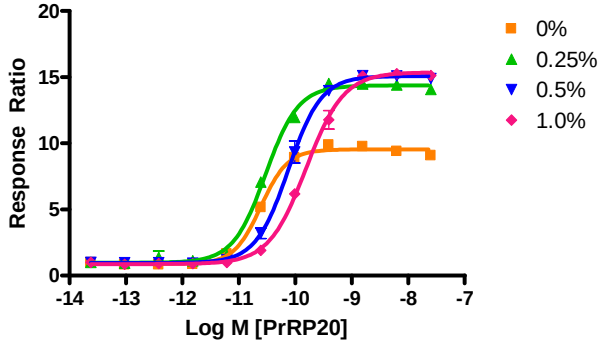
Figure 7 – GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response using 1, 1.5, and 2 hour substrate loading times



GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells were plated at 10,000 cells/well in a 384-well format. Cells were stimulated with a dilution series of PrRP20 (Sigma P7107) 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 cell numbers were obtained using a standard fluorescence plate reader and the Response Ratios plotted against the concentrations of PrRP20 (n=8 for each data point).

Assay Performance with variable DMSO concentration

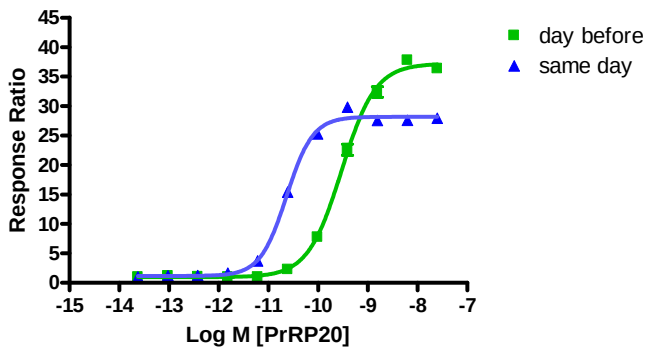
Figure 8 – GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response using 0, 0.25, 0.5 and 1% DMSO.



GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format. Cells were stimulated with a dilution series of PrRP20 (Sigma P7107) for 5 hours. DMSO was added to the assay 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 fluorescence plate reader and the Response Ratios are shown plotted for each DMSO concentration against the concentrations of PrRP20 (n=8 for each data point).

Assay Performance with Various Plating Times

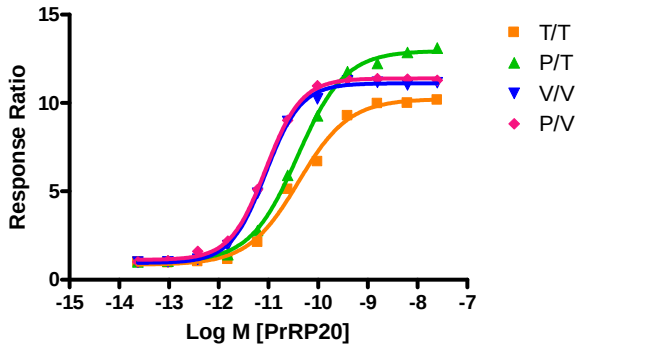
Figure 9- GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response when plated day prior to assay vs same day plating



GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 cells we plated day prior to assay (5,000 cells/well) and day of assay(10,000 cells/well) in a 384-well formatCells were stimulated with a dilution series of PrRP20 (Sigma P7107) for 5 hrs in 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 fluorescence plate reader and the Response Ratios plotted (n=8 for each data point)

Assay Performance with Various Dissociation Methods

Figure 10 – GeneBLAzer® GPR10-NFAT-*bla* CHO-K1 dose response using different dissociation methods



GeneBLAzer® Prior to plating GPR10-NFAT-*bla* CHO-K1 cells (10,000 cells/well) in a 384-well format, they were treated with different wash and dissociation methods. Those conditions included Trypsin Wash/Trypsin dissociation(T/T), PBS wash/Trypsin dissociation(P/T), Versene wash/Versene dissociation(V/V) and PBS wash/Versene dissociation(P/V). Cells were then stimulated with a dilution series of PrRP20 (Sigma P7107) for 5 hrs in 0.5% DMSO 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 plotted (n=8 for each data point).

References

1. Hinuma S, *et al*. **A prolactin-releasing peptide in the brain**. *Nature*. May 21; **393**(6682):272-6. (1998)

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4. Lawrence CB, Ellacot KLJ, Luckman SM. **PRL-Releasing Peptide Reduces Food Intake and May Mediate Satiety Signaling**. *Endocrinology*, **143.2**, 360-367 (2002)

5. Ellacott KLJ, Lawrence CB, Rothwell NJ, Luckman SM. **PRL-Releasing Peptide Interacts with Leptin to Reduce Food Intake and Body Weight**. *Endocrinology* **143.2**, 368-374 (2002)

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