GeneBLAzer® NPSR1-A CHO-K1 DA Cells

GeneBLAzer® NPSR1-A-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1493 and K1477

Cell Line Description

GeneBLAzer® NPSR1-A CHO-K1 DA (Division Arrested) cells and GeneBLAzer®NPSR1-A-NFAT-*bla* CHO-K1 cells contain the human Neuropeptide S Receptor 1—Isoform A A(Accession # NP_997055) 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-cell (NFAT) response element.

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®NPSR1-A CHO-K1 DA cells and GeneBLAzer® NPSR1-A-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations using Neuropeptide S. In addition, GeneBLAzer® NPSR1-A-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 available.

Target Description

Genetic-linkage studies which involved whole genome scanning and genetic mapping of Finnish and Canadian families were performed to identify potential therapeutic targets for asthma and two genes which may confer susceptibility to asthma were identified (1,2). One of these genes encoded a GPCR identified as G protein receptor for asthma susceptibility (GPRA) (1). This GPCR had previously been identified as neuropeptide S receptor (NPSR), vasopression receptor-related receptor 1 (VRR1), and GPR154 (the receptor will be identified as GPRA in the remainder of the document). Prior to its identification as an asthma target, GPRA was shown to be widely expressed in the brain with the highest levels in the hypothalamus, amygdala, endopiriform nucleus, cortex, subiculum, and nuclei of the thalamic midline (10). Stimulation of GPRA in mice resulted in an increase in locomotor activity, wakefulness and anxiolytic-like effects (10).

GPRA was identified as an asthma linked gene in five different Caucasian populations, with GPRA polymorphisms being associated with elevated serum levels of IgE (1,2,3). Two GPRA isoforms, A and B, have been identified, differing in length between 371 (isoform A) and 377 (isoform B) amino acids for GPRA-A and GPRA-B, respectively (1). Both GPRA-A and GPRA-B are expressed in the lung, but expression of GPRA-B is increased in the bronchiolar smooth muscle and epithelial cells of asthmatics when compared to healthy controls (3). When mice asthma models are challenged with an OVA aerosol resulting in airway hyperreactivity to methacholoine, the mRNA levels of GPRA were significantly upregulated (1). A number of single nucleotide polymorphisms of GPRA have been associated with asthma, elevated IgE serum levels, and bronchial hyperresponsiveness, but only one is found in the coding region of the gene, and this single nucleotide polymorphism results in the mutation of amino acid 107 from asparagine to isoleucine (1).

The endogenous ligand of GPRA was a 20- amino acid peptide identified as neuropeptide S (6). Neuropeptide S is cleaved from a larger precursor protein containing a hydrophobic signal peptide and proteolytic processing (10). Expression of the neuropeptide S precursor mRNA has been identified in the brain with the highest levels between the noradrenergic locus coeruleus and Barrington's nucleus in the rat brain stem (10). Neuropeptide S and GPRA mRNA has also been shown to co-localize in the bronchial and colon epithelia (5). Stimulation of both GPRA-A and GPRA-B with neuropeptide S elicits an increase in intracellular cAMP as well as transient increases of intracellular calcium via Gαq and Gαs G-proteins (6). Amino acids 1-6 of neuropeptide S represent the minimal pharmacophore of neuropeptide S required to elicit the GPRA activation to levels seen with the entire 20 amino acid peptide (8,9). The single nucleotide polymorphism which results in the change of amino acid 107 from an asparagines to an isoleucine has been reported as a "gain of function" mutation with increased neuropeptide S potency (7).

Validation Summary

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

1. **Neuropeptide S (NPS) dose response under optimized conditions**

	DA cells	Dividing Cells
EC_{50}	1.4 nM	1.2 nM
Z'-factor	0.71	0.66

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

Assay Testing Summary

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

NPS EC_{50} = 7.1 nM

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

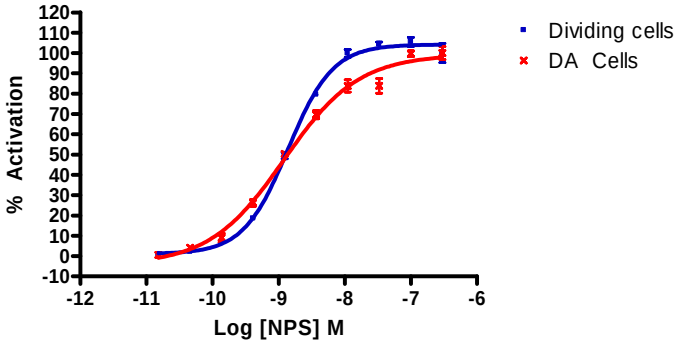
4. **Assay performance with variable stimulation time**

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

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

Primary Agonist Dose Response

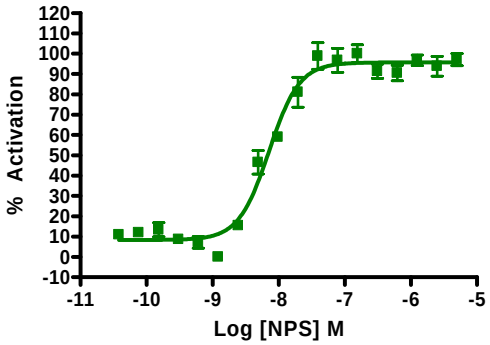
Figure 1 — GeneBLAzer® NPSR1-A CHO-K1 DA and GeneBLAzer® NPSR1-A -NFAT-*bla* CHO-K1 dose response to Neuropeptide S under optimized conditions.



GeneBLAzer® NPSR1-A CHO-K1 DA cells and GeneBLAzer® NPSR1-A-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 Neuropeptide S (NPS) in the presence of 0.1% 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 NPS (n=6 for each data point).

Agonist 2nd Messenger Dose Response

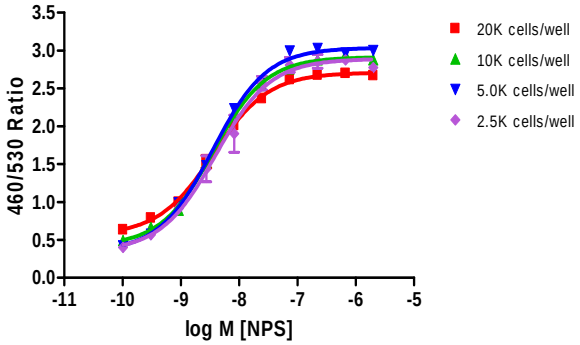
Figure 2— GeneBLAzer® NPSR1-A-NFAT-*bla* CHO-K1 2nd messenger dose response to NPS under optimized conditions.



GeneBLAzer® NPSR1-A-NFAT-*bla* CHO-K1 cells were loaded with Fluo4-AM and tested for a response to NPS.

Assay Performance with Variable Cell Number

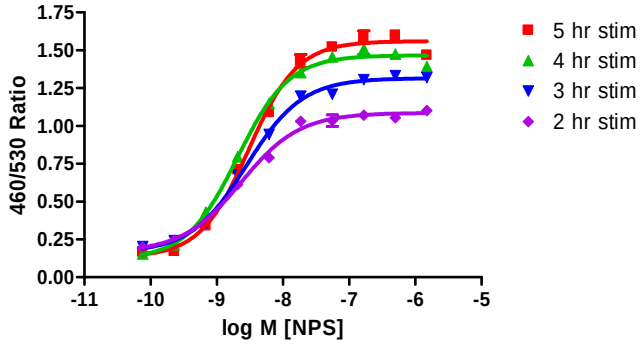
Figure 4 – NPS dose response with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® NPSR1-A-NFAT-*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 neuropeptide S (Phoenix Pharm cat# 005-89) 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 460/530 Emission Ratios are shown plotted for each cell number against the concentrations of NPS (n=8 for each data point).

Assay Performance with Variable Stimulation Time

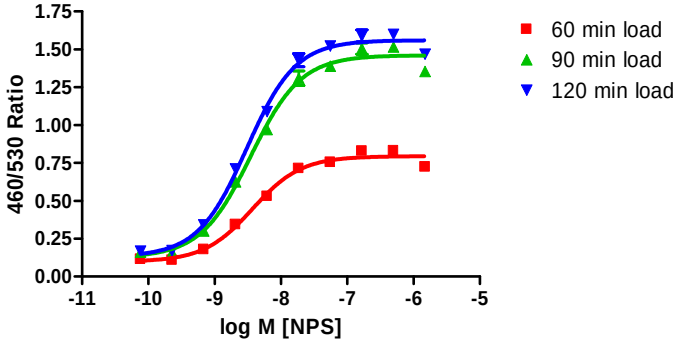
Figure 5 – NPS dose response with 2, 3, 4 and 5 hr stimulation times



GeneBLAzer® NPSR1-A-NFAT-*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 NPS (Phoenix Pharm cat# 005-89) 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 460/530 Emission Ratios are shown plotted for each stimulation time against the concentrations of NPS (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

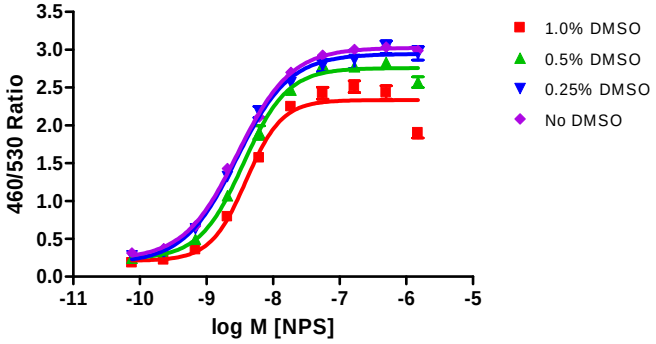
Figure 6 – NPS dose response with 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® NPSR1-A-NFAT-*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 NPS (Phoenix Pharm cat# 005-89) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for either 60, 90, 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 460/530 Emission Ratios are shown plotted for each substrate loading time against the concentrations of NPS (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 7 – NPS dose response with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® NPSR1-A-NFAT-*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 NPS (Phoenix Pharm cat# 005-89) 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 460/530 Emission Ratios are shown plotted for each DMSO concentration against the concentrations of NPS (n=8 for each data point).

References

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