GeneBLAzer® NPSR1-B CHO-K1 DA Assay Kit

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

Catalog Numbers – K1541 and K1481

Cell Line Descriptions

GeneBLAzer® NPSR1-B CHO-K1 DA(Division Arrested) cells and GeneBLAzer ® NPSR1-B-NFAT-*bla* CHO-K1 cells contain the human Neuropeptide S Receptor 1-Isoform B (NPSR1-B), (Accession # NP_997056) 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 NFAT response element. 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® NPSR1-B CHO-K1 DA cells and GeneBLAzer® NPSR1-B-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of Neuropeptide-S (Figure 1). In addition, GeneBLAzer® NPSR1-B-NFAT-*bla* CHO-K1 cells have been tested for assay performance under variable conditions.

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 (4). Stimulation of GPRA in mice resulted in an increase in locomotor activity, wakefulness and anxiolytic-like effects (4).

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).

Validation Summary

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

1. Neuropeptide-S dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	18.5 nM	14.6 nM
Z'-factor	0.7479	

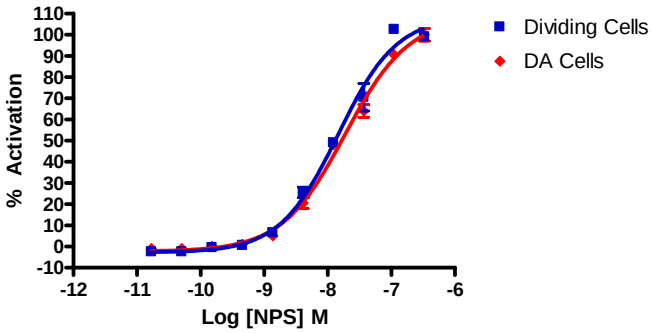
Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 333 nM

Assay Testing Summary

1. Assay performance in 2nd messenger assay

Primary Agonist Dose Response

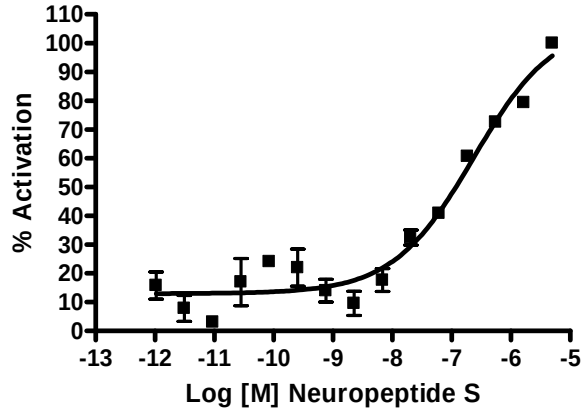
Figure 1 — GeneBLAzer® NPSR1-B CHO-K1 DA and GeneBLAzer® NPSR1-B-NFAT-*bla* CHO-K1 cells dose response to Neuropeptide-S under optimized conditions



GeneBLAzer® NPSR1-B CHO-K1 DA cells and GeneBLAzer® NPSR1-B-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 (Phoenix Pharm 005-89) 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 Neuropeptide-S.

2nd Messenger Dose Response

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



GeneBLAzer® NPSR1-B-NFAT-*bla* CHO K1 cells were loaded with Fluo4 Direct™ and tested for a response to Neuropeptide S.

References

1) Laitinen, T., Polvi, A., Rydman, P., Vendelin, J., Pulkien, V., Salmikangas, P., Makela, S., Rehn, M., Pirskanen, A., Rautanen, A., Zucchelli, M., Gullsten, H., Leino, M., Alenius, H., Petays, T., Haahtela, T., Laitinen, A., Laprise, C., Hudson, TJ., Laitinen, LA., and Kere, J. (2004) *Science*. **304**, 300-304.
2) Laitinen, T., Daly, MJ., Rioux, JD., Kauppi, P., Laprise, C., Petays, T., Green, T., Cargill, M., Haahtela, T., Lander, ES., Laitinen, LA., Hudson, TJ., and Kere, J. (2001) *Nature Genetics*. **28**, 87-91.
3) Kormann, MSD., Carr, D., Klopp, N., Illig, T., Leupold, W., Fritzsch, C., Weiland, SK., von Mutius, E., and Kabesch, M. (2005) *American Journal of Critical Care Medicine*. **171**, 1358-1362.
4) Xu, YL., Reinscheid, RK., Huitron-Resendiz, S., Clark, SD., Wang, Z., Lin, SH., Brucher, FA., Zeng, J., Ly, NK., Henriksen, SJ., de Lecea, L., and Civelli, O. (2004) *Neuron*. **43**, 487-497.