

Optimization of the GeneBLAzer® NTSR1-NFAT-bla CHO-K1 Cell Line

GeneBLAzer[®] NTSR1 CHO-K1 DA Cells

GeneBLAzer[®] NTSR1-NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1773 and K1781

Cell Line Descriptions

GeneBLAzer[®] NTSR1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer[®] NTSR1-NFAT-*bla* CHO-K1 cells contain the human Neurotensin Receptor 1 (NTSR1), (Accession # NM_002531) stably integrated into the CellSensor[®] NFAT-bla CHO-K1 cell line. CellSensor[®] NFAT-bla CHO-K1 cells (Cat. no. K1534) contain a beta-lactamase reporter gene under control of the NFAT. 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[®] NTSR1 CHO-K1 DA cells and GeneBLAzer[®] NTSR1-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC₅₀ concentrations of Neurotensin (Figure 1).

Validation Summary

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

1. Neurotensin dose response under optimized conditions

| | DA cells | Dividing Cells |
|----------------------------|----------|----------------|
| EC ₅₀ | 0.185 nM | 0.557 nM |
| Z'-factor | 0.81 | 0.67 |
| | | |
| Recommended cell no. /well | | = 10,000 |
| Recommended Stim. Time | | = 5 hrs |
| Max. [Stimulation] | | = 250 nM |
| - | - | |

Primary Agonist Dose Response

Figure 1 — GeneBLAzer[®] NTSR1-NFAT-*bla* CHO-K1 and GeneBLAzer[®] NTSR1-NFAT-*bla* CHO-K1 DA response to Neurotensin under optimized conditions



GeneBLAzer[®] NTSR1-NFAT-*bla* CHO-K1 10,000 cells/well) and NTSR1-NFAT-CHO-K1 DA cells plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of Neurotensin (Sigma N6383) 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 Neurotensin.