GeneBLAzer® NMUR1 CHO-K1 DA Assay Kit

GeneBLAzer® NMUR1 NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1331 and K1719

Cell Line Description

GeneBLAzer® NMUR1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 cells contain the human neuromedin U-25 receptor 1 (NMUR1), (Accession # NM_006056.2)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® NMUR1 CHO-K1 DA cells and GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of neuromedin U-25 (Figure 1). In addition, GeneBLAzer® NMUR1-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 (data included upon request). Additional testing data using alternate stimuli are also included.

Target Description

Neuromedin U was first purified in 1985 from porcine spinal chord and named for its ability to induce uterine contractions. Two forms of the protein were identified, a 25 amino acid long peptide and an 8 amino acid long peptide which is identical to the C-terminus of the larger peptide (1-2). In humans, only neuromedin U-25 is present. The amino acid sequence of neuromedin U is remarkably conserved between species (reviewed in 3).

Two receptors for Neuromedin U have been identified through various studies and are 50% homologous to each other (4-6).The receptors have different patterns of expression. NMUR1 localizes to the peripheral organs including the gastrointestinal tract, urogenital system and kidneys. The CNS was found to express much lower amounts of NMUR1 than the periphery organ systems. Contrastingly, NMUR2 is expressed primarily in the CNS (6). Both receptors are believed to couple to the Gq family of proteins upon ligand binding (reviewed in 3).

The physiological roles for these receptors are unknown. Some recent animal studies have implicated NMUR1 to be involved in inflammation (7), regulation of body weight, and feeding (8).

Validation Results

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

1. Neuromedin U-25 agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	1 nM	3 nM
Z'-factor	0.81	0.73

Recommended cell no. = 2.5K cells/well
Recommended [DMSO] = up to 1%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = 3.2 µM

2. Alternate agonist dose response

Neuromedin U-8 EC_{50} = 1.1 nM

3. Antagonist dose response

No commercial sources of antagonist were available for testing at the time of publication of this document

Assay Performance with Variable Conditions

4. Assay performance with variable cell number

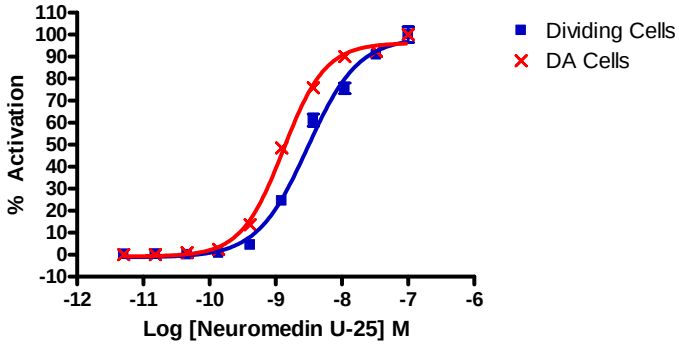
5. Assay performance with variable stimulation time

6. Assay performance with variable substrate loading time

7. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

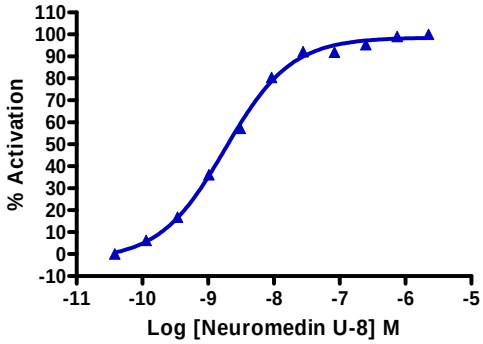
Figure 1 — GeneBLAzer® NMUR1 CHO-K1 DA and GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 dose response to neuromedin U-25 under optimized conditions



GeneBLAzer® NMUR1 CHO-K1 DA cells and NMUR1-NFAT-*bla* CHO-K1 cells (2,500 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of neuromedin U-25 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 % Activation plotted for each replicate against the concentrations of neuromedin U-25 (n=6 for each data point).

Alternate Agonist Dose Response

Figure 2 — GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 dose response to Neuromedin U-8 under optimized conditions

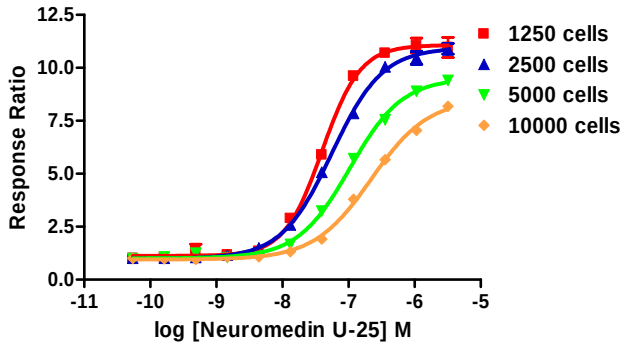


GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 cells (2,500 cells/well) were assayed on three separate days. Cells were plated the day before the assay in a 384-well format and stimulated with Neuromedin U-8 (Phoenix Pharmaceuticals #046-39) over the indicated concentration range in the presence of 0.5% DMSO for 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 % Activation plotted against the indicated concentrations of Neuromedin U-8 (n=16 for each data point).

Note: The Neuromedin U-25 used for the following validation experiments was stored for over 1 year and was not as active as the fresh Neuromedin U-25 used for the experiments shown in the primary agonist section leading to a difference in potency.

Assay Performance with Variable Cell Number

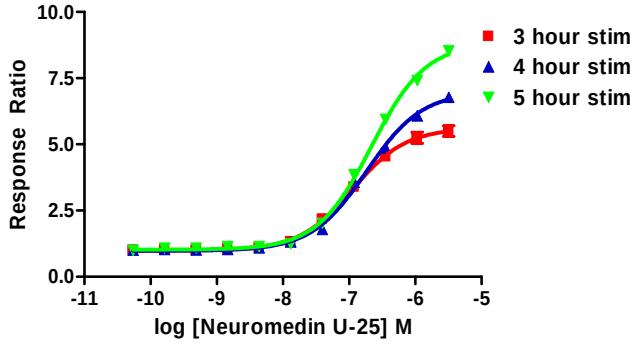
Figure 3— GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 dose response to Neuromedin U-25 using 1.25, 2.5, 5, and 10K cells / well



GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 cells were plated the day before the assay at 1,250 2,500 or 5,000 and 10,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with Neuromedin U-25 (Sigma # N8138) 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 for each cell number against the indicated concentrations of Neuromedin U-25 (n=8 for each data point).

Assay Performance with Variable Stimulation Time

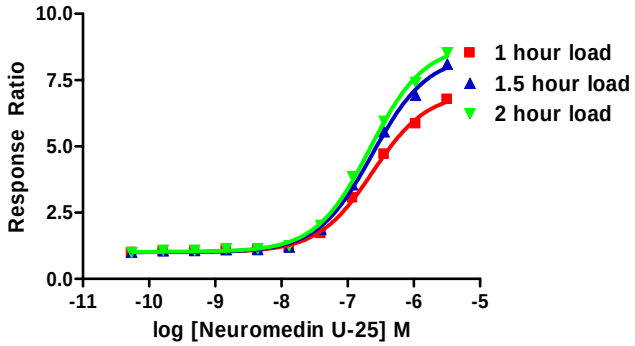
Figure 4 – GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 dose response to Neuromedin U-25 with 3, 4 and 5 hr stimulation times



GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. Neuromedin U-25 (Sigma # N8138) was then added to the plate over the indicated concentration range for 3, 4, or 5 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 plotted against the indicated concentrations of Neuromedin U-25 (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

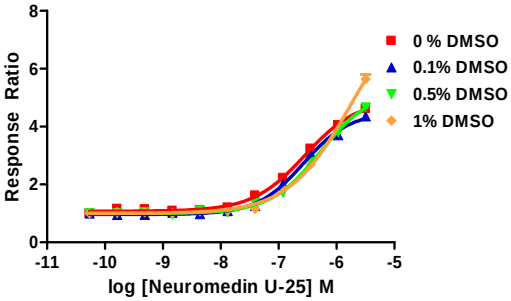
Figure 5— GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 dose response to Neuromedin U-25 with 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well black-walled tissue culture assay plate. Neuromedin U-25 (Sigma # N8138) was then added to the plate over the indicated concentration range in 0.5% DMSO for 5 hours and then loaded for 1, 1.5 or 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 plotted against the indicated concentrations of Neuromedin U-25 (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 6 – GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 dose response to Neuromedin U-25 with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® NMUR1-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. Neuromedin U-25 (Sigma # N8138) was then added to the plate over the indicated concentration range. DMSO was added to separate wells 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 shown plotted for each DMSO concentration against the indicated concentrations of Neuromedin U-25 (n=8 for each data point).

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