GeneBLAzer® M3 NFAT-*bla* CHO-K1 Cells

Catalog Numbers – K1716

Cell Line Descriptions

GeneBLAzer®M3-NFAT-*bla* CHO-K1 cells contain the human Acetylcholine (muscarininc) subtype 3 receptor (M3), (Accession# NM_000740.2) 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.

The GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of carbachol, (Figure 1). In addition, GeneBLAzer® M3-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

Muscarinic acetylcholine receptors are members of the G protein-coupled receptor (GPCR) superfamily and are widely distributed and mediate the actions of acetylcholine in both the CNS and peripheral tissues. Five muscarinic receptor subtypes have been identified and are referred to as M_1-M_5 (1-5). The five genes that encode the muscarinic receptors all belong to the rhodopsin-line family (Family A) and share strong sequence homology, but have unique regions located at the amino terminus (extracellular) and in the third intracellular loop.

The M_1, M_3, and M_5 receptor subtypes couple through the $G_{q/11}$ class of G-proteins and activate the phopholipase C pathway. In smooth muscle, activation of the M_3 receptor leads to contraction and in glandular tissue, M_3 activation leads to hormonal secretion. In brain, M_3 activation mediates "slow" neuronal excitability. Cortical and hippocampal muscarinic receptors are thought to be important in the attentional aspects of cognition. The predominant receptor subtypes in these brain areas are M_1, M_3, and M_4. Studies on knock-out mouse models of M_3 are also beginning to reveal additional potential functions of the receptor (6-11). Additional information on the muscarinic receptors can be found in reviews (12-15). M_3 may play an important role for cognition, chronic obstructive pulmonary disease, urinary incontinence, and obesity (6-11).

Validation Summary

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

1. Carbachol agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	1.0 µM	2.6 µM
Z'-factor	0.88	0.71

Optimum cell no.	= 20K cells/well
Optimum [DMSO]	= up to 1%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 100 µM

2. Alternate agonist dose response

Bethanecol EC_{50}	= 31 µM
Oxotrmorine M EC_{50}	= 0.4 µM
MCN–A-343 EC_{50}	= 5 µM
Pilocarpine EC_{50}	= 1.6 µM

3. Antagonist dose response

Telenzipine IC_{50}	= 12 nM
Scopolamine M IC_{50}	= 154 pM
DAMP IC_{50}	= 973 pM
Methoctramine IC_{50}	= 6.9 µM

4. Agonist dose response using Fluo-4NW

Carbachol EC_{50}	= 300 nM

Assay Testing Summary

5. Assay performance with variable cell number

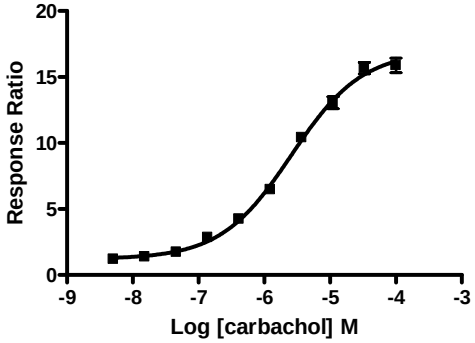
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

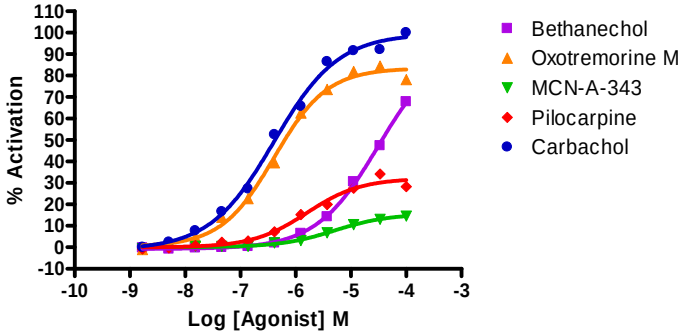
Figure 1 — GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response to carbachol under optimized conditions



GeneBLAzer® M3-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 carbachol 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 carbachol (n=6 for each data point).

Alternate Agonist Dose Response

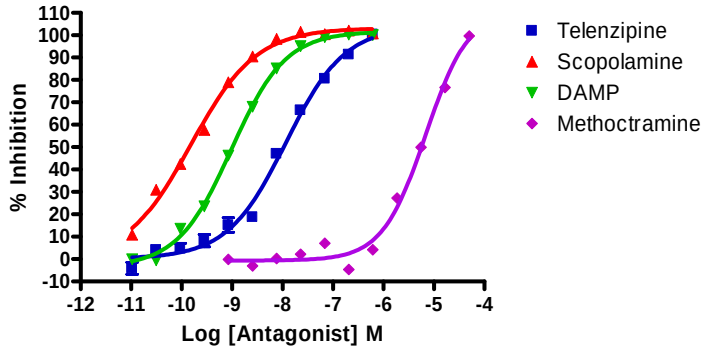
Figure 2 — GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response to Bethanecol, Oxotremorine M, MCN-A-343, Pilocarpine and Carbachol



GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells (20,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with Carbachol (Sigma #21760), Bethanechol (Sigma #C5259), Oxotremorine (Sigma # O-100), MCN-A-343(Sigma # C7041), or Pilocarpine (Sigma # P6503) 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 the % Activataion plotted against the indicated concentrations of the agonists (n= 8 for each data point).

Antagonist Dose Response

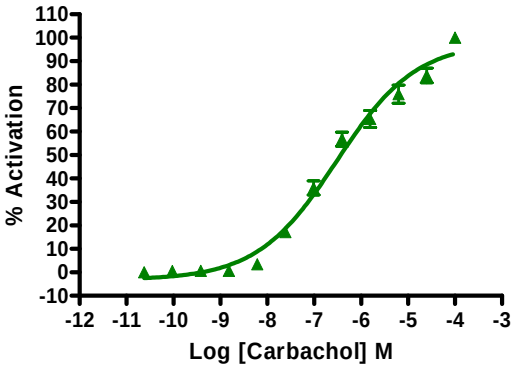
Figure 3 — GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response to Telenzipine, Scopolamine, DAMP and Methoctramine



GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells (20,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Cells were treated with the antagonists Telenzipine (Sigma # T-122), Scoploamine (Sigma #S1875), DAMP (Sigma #D-104) or Methoctramine (Sigma # M-105) and incubated at 37 degrees C for 20 min., followed by 1.5 µM Carbachol agonist stimulation for 5 hours in 0.5% DMSO. Cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate 2µM final concentration of CCF4-AM + 1mM Solution D). Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of the antagonists (n=8 for each data point).

Agonist Dose Response Using Fluo-4NW

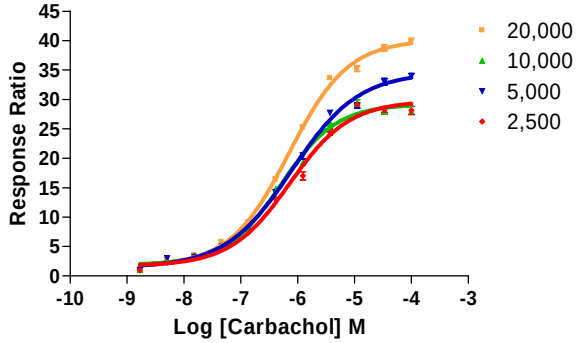
Figure 4 — GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response to Carbachol using Fluo-4NW



GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format. Cells were then incubated with Fluo-4NW for 30 min. at 37°C, followed by 30 min. at room temperature. Cells were then stimulated with a dilution series of Carbachol (Sigma #21760) in the presence of 0.5% DMSO. Fluorescence emission values at 516 nm were obtained and the % Activation was plotted against the indicated concentrations of agonist (n=16 for each data point).

Assay Performance with Variable Cell Number

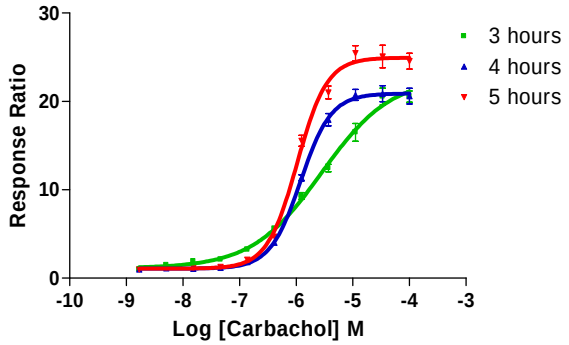
Figure 4— GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response using 2.5, 5, 10, and 20K cells/well



GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells were plated the day of the assay at 2500, 5000, 10,000 or 20,000 cells/well in a 384-well format. Cells were then stimulated with Carbachol (Sigma #21760) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (2µM final concentration of CCF4-AM + 1mM Solution D) 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 indicated concentrations of Carbachol (n=8 for each data point).

Assay performance with Variable Stimulation Time

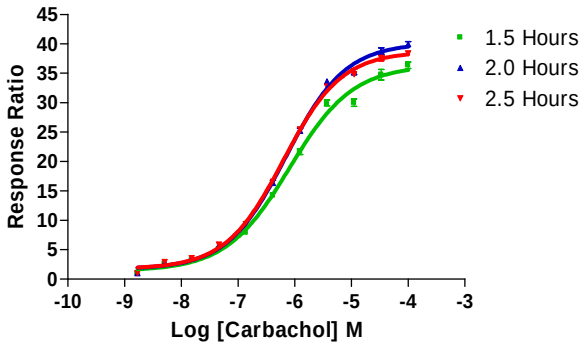
Figure 5 – GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response using 3, 4, and 5 hour stimulation times



GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells (20,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Carbachol (Anaspec #22872) 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 (2µM final concentration of CCF4-AM + 1mM Solution D). 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 Variable Substrate Loading Time

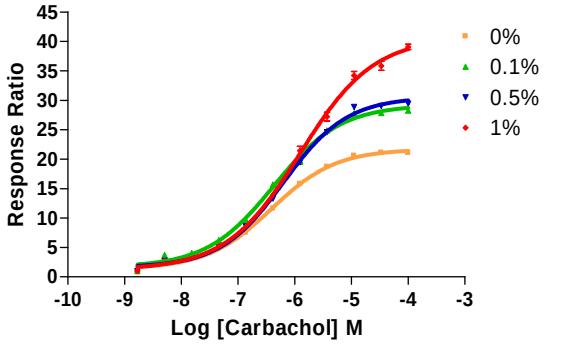
Figure 6 – GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response using 1.5, 2.0 and 2.5 hour substrate loading times



GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells were plated the day of the assay at 20,000 cells/well in a 384-well format. Cells were then stimulated with Carbachol (Sigma #21760) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate (2µM final concentration of CCF4-AM +1mM Solution D) for either 1.5, 2 and 2.5 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 indicated concentrations of Carbachol (n=8 for each data point).

Assay Performance with variable DMSO concentration

Figure 7 – GeneBLAzer® M3-NFAT-*bla* CHO-K1 dose response using 0, 0.1, 0.5 and 1% DMSO.



GeneBLAzer® M3-NFAT-*bla* CHO-K1 cells (10,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Carbachol (Anaspec #22872) was then added to the plate over the indicated concentration range. DMSO was added to the assay 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 (2µM final concentration of CCF4-AM + 1mM Solution D). 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 carbachol (n=8 for each data point).

References

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