GeneBLAzer® M1 NFAT-*bla* Jurkat Cells

Catalog Numbers – K1710

Cell Line Descriptions

GeneBLAzer® M1-NFAT-*bla* Jurkat cells contain the human Acetylcholine (muscarinic) subtype 1 receptor (M1), (Accession #NM_000738) stably integrated into the CellSensor® NFAT-*bla* Jurkat cell line. CellSensor® NFAT-*bla* Jurkat cells (Cat. no. K1671) contain a beta-lactamase (*bla*) reporter gene under control of the Nuclear Factor of Activated T-cells (NFAT) response element.

M1-NFAT-*bla* Jurkat cells are functionally validated for Z'-factor and EC_{50} concentrations of carbachol (Figure 1). In addition, GeneBLAzer® M1-NFAT-*bla* CHO-K1 cells have been tested for assay performance under variable conditions.

Target Description

Muscarinic acetylcholine receptors are members of the G protein-coupled receptor (GPCR) superfamily. Muscarinic receptors 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. Activation of this pathway in turn leads to increases in free intracellular calcium levels as inositol triphosphate mediates release of calcium from the endoplasmic reticulum. In addition, protein kinase C is activated via diacylglycerol. The M_2 and M_4 receptor subtypes couple through the $G_{i/o}$ class of G proteins and inhibit adenylyl cyclase activity.

In the brain, M_1 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. Therefore, M_1 is a potential target for cognition, Alzheimer's, dementia, and schizophrenia (6). Studies on knock-out mouse models of M_1 are also beginning to reveal potential functions of the receptor (7-9). Additional information on the muscarinic receptors can be found in reviews (10-13).

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

Dividing Cells
EC_{50} = 730 nM
Z'-factor = 0.95

Optimum cell no. = 25K cells/well
Optimum [DMSO] = up to 1%
Optimum Stim. Time = 5 hours
Max. [Stimulation] = 100 µM

2. Alternate agonist dose response

MCN –A-343 EC_{50} = 2.7 µM
Pilocarpine EC_{50} = 845 nM

3. Antagonist dose response

Telenzipine IC_{50} = 10 nM
Scopolamine IC_{50} = 2.2 µM
Methoctramine IC_{50} = 3.1 µM

4. Agonist 2nd messenger dose response

5. [^{3}H] NMS saturation binding analysis

K_D [^{3}H] NMS = 0.08 nM
B_{max} (pmol/mg) = 4.5

6. Competitive [^{3}H] NMS binding analysis to determine K_i values

Ligand	K_i (nM)	Literature K_i (nM) *
Scopolamine	0.23	1
Atropine	0.45	0.2 – 3.2
Pirenzepine	6.8	5 – 500
Himbacine	83	79 - 200

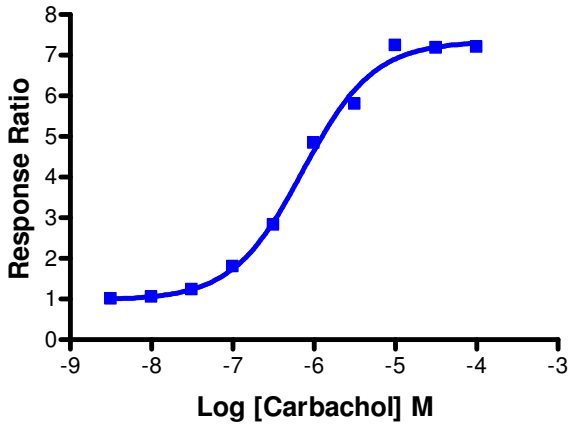
* Literature K_i values were obtained from International Union of Pharmacology (IUPHAR) reference database (14).

7. Agonist-induced [^{35}S] GTPγS binding

Ligand	EC_{50} (µM)	E_{max}
Acetylcholine	1.1	102%
Methacholine	0.86	101%
Oxotremorine M	0.51	91%
Carbachol	5.6	105%
McN-A-343	0.36	21%
Bethanechol	11	39%

Primary Agonist Dose Response

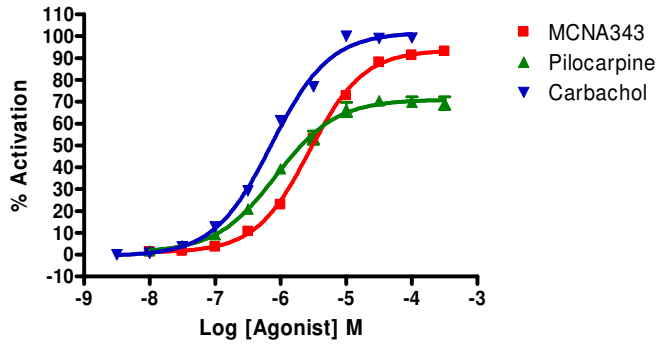
Figure 1 — GeneBLAzer® M1-NFAT-*bla* Jurkat dose response to carbachol under optimized conditions



GeneBLAzer® M1-NFAT-*bla* Jurkat cells (25,000 cells/well) were plated in a 384-well format and stimulated with Carbachol (Sigma #21760) 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 % Activation plotted against the indicated concentrations of carbachol

Alternate Agonist Dose Response

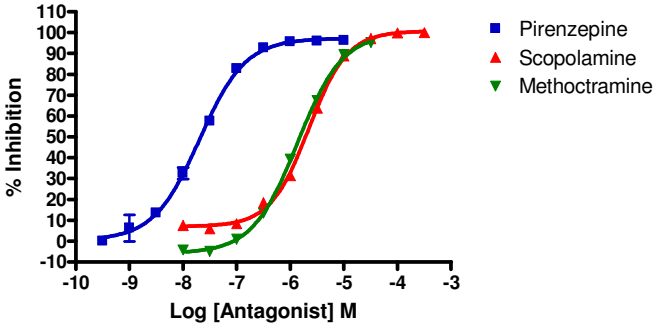
Figure 2 — GeneBLAzer® M1-NFAT-*bla* Jurkat dose response to MCN-A-343, Pilocarpine and Carbachol



GeneBLAzer® M1-NFAT-*bla* Jurkat cells (25,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with either 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 % Activation plotted against the indicated concentrations of the agonists.

Antagonist Dose Response

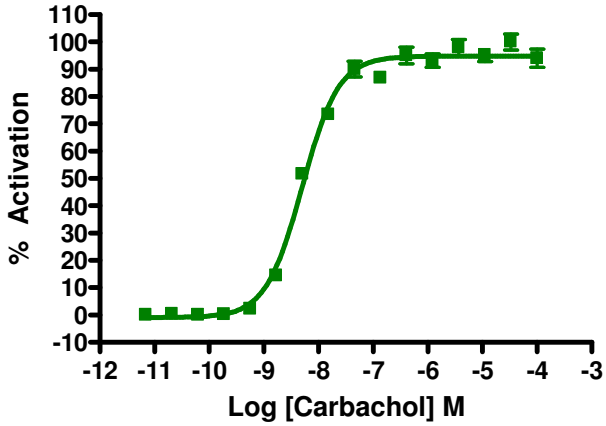
Figure 3 — GeneBLAzer® M1-NFAT-*bla* Jurkat dose response to Perenzipine, Scopolamine, and Methoctramine



GeneBLAzer® M1-NFAT-*bla* Jurkat cells (25,000 cells/well) were plated the day of the assay in a 384-well black-walled tissue culture assay plate. Cells were treated with Pirenzipine (P7412), Scopolamine (Sigma #S1875), or Methoctramine (Sigma #M-105) for 30 minutes prior to incubation with an EC80 concentration of Carbachol agonist for 5 hours in 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 fluorescence plate reader and the % Inhibition is shown plotted against the indicated concentrations of the antagonists. The data shows the correct rank order potency.

Agonist 2nd Messenger Dose Response

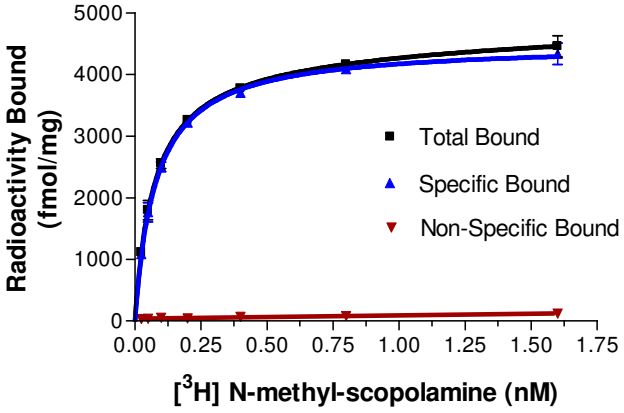
Figure 4 — GeneBLAzer® M1-NFAT-*bla* Jurkat dose response to Carbachol



GeneBLAzer® M1-NFAT-*bla* Jurkat cells were loaded with Fluo4-AM and tested for a response to Carbachol.

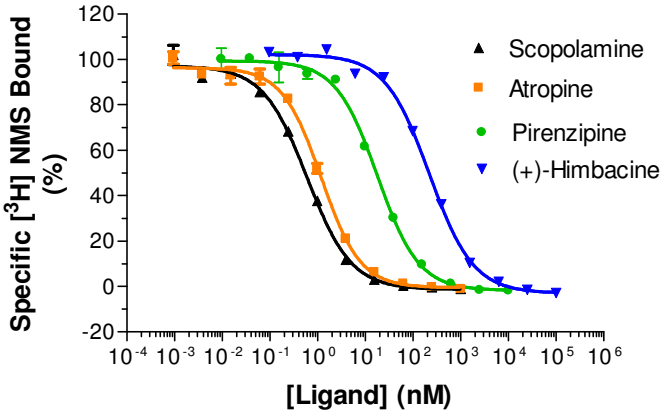
Radioligand Binding and Competition

Figure 5 — [^{3}H] NMS saturation binding to GeneBLAzer® M1-NFAT-*bla* Jurkat Membranes



Saturation binding analysis was performed by incubating GeneBLAzer® M1-NFAT-*bla* Jurkat membranes with increasing concentrations of [^{3}H] N-methyl-scopolamine (NMS) in PBS, pH 7.4, with 100 µg/ml BSA. Non-specific binding was determined in the presence of 10 µM atropine. Samples were incubated for 2 hrs prior to filtration through a GF/B 96-well filter plate, which was pre-treated with 0.5% PEI. Filters were washed with cold 50 mM Tris-Cl, pH 7.4.

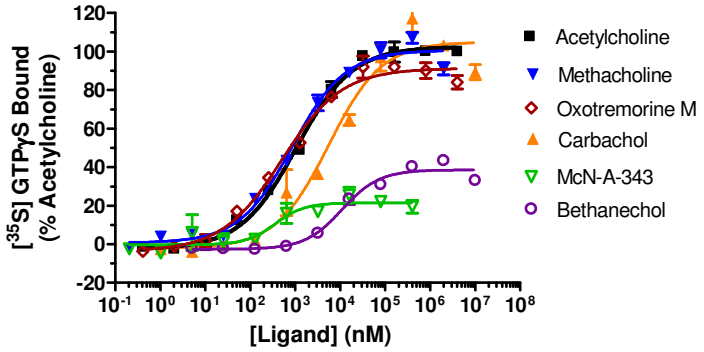
Figure 6 — Competitive Radioligand binding to to GeneBLAzer® M1-NFAT-*bla* Jurkat Membranes



Competitive radioligand binding analysis was performed by incubating GeneBLAzer® M1-NFAT-*bla* Jurkat membranes, cold competitive ligands, and [^{3}H] N-methyl-scopolamine (NMS) in 20 mM Hepes, pH 7.5, 20 mM NaCl, 5 mM $MgCl_2$, with 40 µg/ml BSA. Samples were incubated for 2 hrs prior to filtration through a GF/B 96-well filter plate, which was pre-treated with 0.5% PEI. Filters were washed with cold 50 mM Tris-Cl, pH 7.4, 5 mM $MgCl_2$. Data was converted to be a percent of radioactivity bound such that binding in the presence or absence of 5 µM atropine is equivalent to 0% and 100%, respectively.

Agonist induced [^{35}S] GTPγS Binding

Figure 7 — Agonist-induced [^{35}S] GTPγS binding to GeneBLAzer® M1-NFAT-*bla* Jurkat Membranes



[^{35}S] GTPγS Binding analysis was performed by incubating GeneBLAzer® M1-NFAT-*bla* Jurkat membranes with agonists, 0.20 nM [^{35}S]-GTPγS, 20 mM Hepes, pH 7.5, 20 mM NaCl, 5 mM $MgCl_2$, 3 µM GDP, 10 µg/ml Saponin, 1 mM EGTA. Samples were filtered through a GF/C 96-well filter plate and washed with cold 50 mM Tris-Cl, pH 7.4, 5 mM $MgCl_2$. Data was converted to percent [^{35}S] GTPγS bound where 100% and 0% represent binding in the presence and absence of 4 mM acetylcholine,respectively.

References

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