GeneBLAzer® ADORA2A CHO-K1 DA Assay Kit

GeneBLAzer® ADORA2A CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1345 and K1530

Cell Line Descriptions

GeneBLAzer® ADORA2A CHO-K1 DA (Division Arrested) cells and GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells contain the human Adenosine A_{2A} (ADORA2A) receptor (Accession # NM_000675) stably integrated into the CellSensor® CRE-*bla* CHO-K1 cell line. CellSensor® CRE-bla CHO-K1 cells (Cat. no. K1534) contain a beta-lactamase (*bla*) reporter gene under control of the Cyclic AMP Response Element (CRE). 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® ADORA2A CHO-K1 DA cells and GeneBLAzer® ADORA2A-CRE-bla CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of 5'-(N-Ethylcarboxamido) adenosine (NECA). In addition, GeneBLAzer® ADORA2A-CRE-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

Adenosine receptors are a class of GPCRs that respond to the purine Adenosine (3). The discovery of two different classes of adenosine receptors came when adenosine derivatives either increased or decreased intracellular cAMP (1-2). Receptors that decreased intracellular cAMP were named A_1 and those that increased intracellular cAMP were named A_2 receptors (3-4). The separation of the A_2 classes was due to the differences in affinity to adenosine (5). Receptors with high affinity (0.1-1 µM) to adenosine were classified A_{2A} and those with low affinity (10µm) were classified A_{2B} (5).

Adenosine A_{2A} Receptor (ADORA2A) has been shown to have many roles throughout the body. ADORA2A is involved in pain and inflammation regulation, inhibition of platelet aggregation, blood pressure regulation and contributes to ischemic brain damage (7). Selective antagonists of the ADORA2A pathway are being tested as adjuvants to dopaminergic drugs to treat Parkinson's disease and schizophrenia (7).

ADORA2A receptors are found with various expression levels throughout the body. Expression is highest in spleen, thymus, leukocytes and the olfactory bulb. Heart, blood vessels and lung contain receptors with intermediate expression. Low levels are found in other regions of the brain (7).

Validation Summary

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

1. **Primary agonist dose response under optimized conditions(n=3)**

	DA cells	Dividing Cells
(NECA)EC_{50}	5 nM	8 nM
Z'-factor	0.71	0.63

Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 0.5%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 25µM

2. **Alternate agonist dose response**

CGS 21680 (EC_{50})	= 13 nM
Adenosine (EC_{50})	= 226 nM

3. **Antagonist dose response**

ZM241385 (IC_{50})	= 14.9 nM
SCH58261 (IC_{50})	= 21.5 nM

4. **Agonist 2nd messenger dose response**

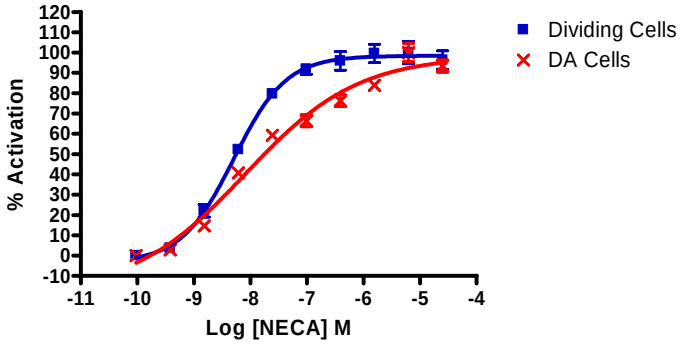
NECA (EC_{50})	= 5.1 nM

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

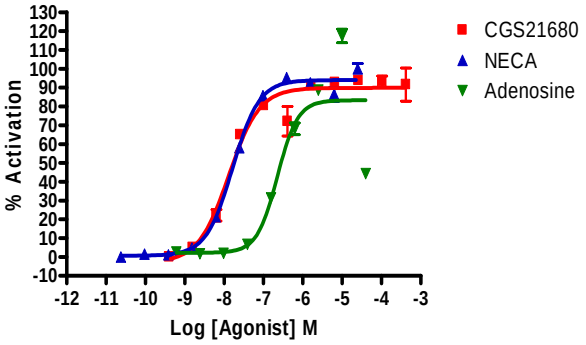
Figure 1 — GeneBLAzer® ADORA2A DA Cells and ADORA2A-CRE-*bla* CHO-K1 dose response to NECA under optimized conditions



GeneBLAzer® ADORA2A CHO-K1 DA cells and GeneBLAzer® ADORA2A-CRE-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 5' adenosine (NECA) 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 plotted for each replicate against the concentrations of NECA (n=6 for each data point).

Alternate Agonist Dose Response

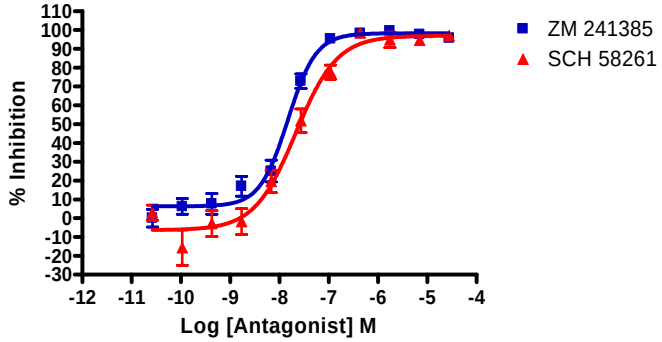
Figure 2 — GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 dose response to NECA, Adenosine and CGS 21680



GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with dilution series of NECA (Sigma E2387), Adenosine (Sigma A4036) and CGS 21680 (Tocris 1063) 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 florescence plate reader and the 460/530 Emission Ratios are shown plotted against the concentrations of NECA and Adenosine (n=8 for each data point). The data shows the correct rank order potency as NECA and CGS21680 are equipotent (6). Adenosine is significantly less potent than other agonists (7).

Antagonist Dose Response

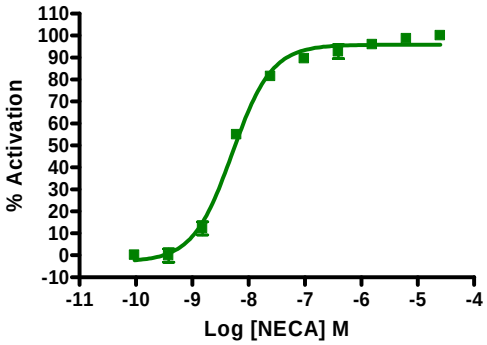
Figure 3 — GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 dose response to ZM 241385 and SCH 58261



GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells were plated 16-20 hours prior to assay at 10,000 cells per well in a 384-well format. Cells were treated with a dilution series of SCH 58261 (Tocris #2270) and ZM 241685 (Tocris #1036) in the presence of 0.25% DMSO. Cells were incubated at 37ºC & 5% CO_2 for 30 min. NECA (Sigma #E2387) was added to the plate at the EC_{80} concentration of 44.0 nM along with 0.25% DMSO (0.5% Final concentration). Cells were incubated for 5 hours and loaded for2 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 shown plotted against the concentrations of the antagonists. The data

Agonist 2nd Messenger Dose Response

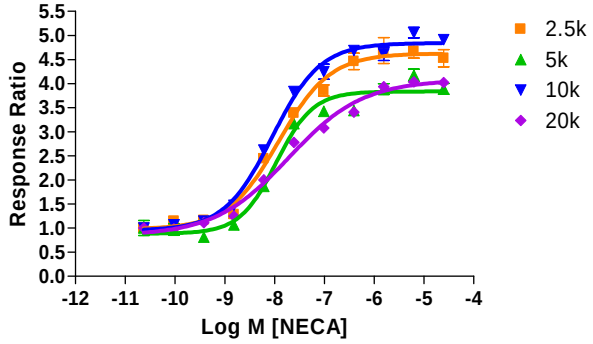
Figure 4— GeneBLAzer® ADORA2A-CRE-*bla* CHO-k1 2nd messenger dose response to NECA under optimized conditions.



GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells were tested for a response to NECA with a TR-FRET cAMP assay

Assay Performance with Variable Cell Number

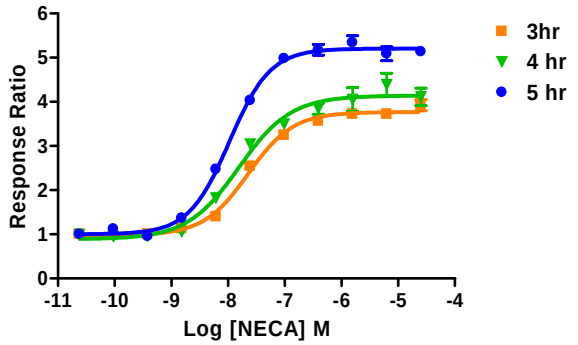
Figure 5 – GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 dose response to NECA with 2.5, 5, 10, and 20K cells / well



GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells were plated at 2,500 5,000 10,000 or 20,000 cells/well in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of NECA (Sigma #E2387) 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 and the Response Ratios for each cell number plotted against the concentrations of NECA (n=8 for each data point).

Assay Performance with Variable Stimulation Time

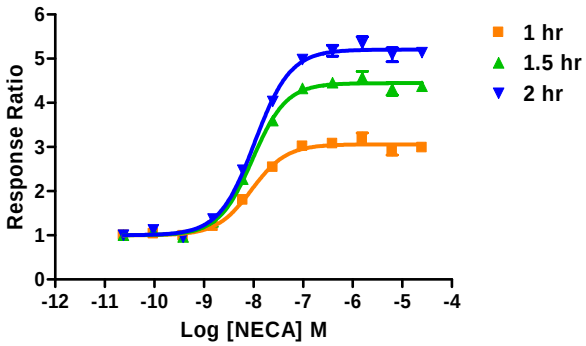
Figure 6 – GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 dose response to NECA with 3, 4 and 5 hr stimulation times



GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of NECA (Sigma #E2387) for 3, 4, or 5 hrs in the presence of 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 florescence plate reader and the Response Ratios for each stimulation time plotted against the concentrations of NECA (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

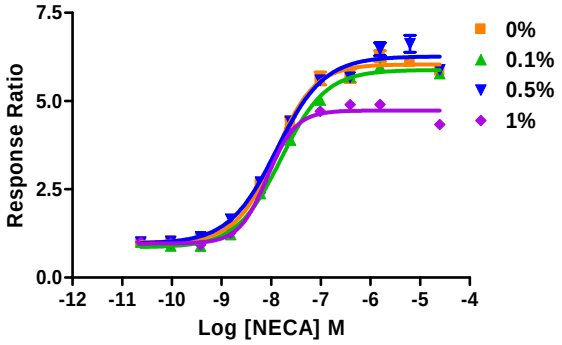
Figure 7 – GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 dose response to NECA with 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of NECA (Sigma #E2387) in the presence of 0.5% DMSO for 5 hours. Cells were then loaded for either 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 florescence plate reader and the Response Ratios for each substrate loading time plotted against the concentrations of NECA (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 dose response to NECA with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® ADORA2A-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Prior to assay the plating media was removed and assay media added. Cells were stimulated with a dilution series of NECA (Sigma #E2387) for 5 hours. DMSO was added to the cells at concentrations from 0% to 1%. 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 florescence plate reader and the Response Ratios for each DMSO concentration plotted against the concentrations of NECA (n=8 for each data point).

References

1. Van Calker D. et al. **Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells**. *J. Neurochem.* **33**, 999-1005 (1979).

2. Londos, C. et al. **Subclasses of external adenosine receptors**. *Proc. Natl.Acad. Sci. U.S.A*. **77**, 2551-2254 (1980).

3. Fredholm, B.B., et al. **Nomenclature and classification of purinoceptors**. *Pharmacol. Rev.* **46**, 143-156. (1994).

4. Fredholm, B.B., et al. **Nomenclature and classification of adenosine receptors.** *Pharmacol. Rev.* **53**, 527-552. (2001).

5. Daly, J.W. et al. **Subclass of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines.** *Cell Mol. Neurobiol.*. **3**, 69-80 (1993).

6. Villa de Brito, M.T. et al. **Adenosine A2A receptors in portal hypertension: their role in the abnormal response to adenosine of the cranial mesenteric artery in rabbits.** *Br. J. of Pharmacology.* **135**, 1324-1330 (2002).

7. Fredholm, B.B. et al. **Pharmacology of Adenosine A_{2A} Receptors and Therapeutic Applications.** *Current Topics in Medicinal Chemistry*, **3,** 1349-1364 (2002).