GeneBLAzer® D1 CHO-K1 DA Assay Kit

GeneBLAzer® D1 CRE-*bla* CHO-K1 Cells

Catalog Numbers – K1311 and K1709

Cell Line Descriptions

GeneBLAzer® D1 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® D1-CRE-*bla* CHO-K1 cells contain the human dopamine receptor 1 (D1) (Accession # NM_000794.2) stably integrated into the CellSensor® CRE-*bla* CHO-K1 cell line. CellSensor® CRE-*bla* CHO-K1 cells (Cat. no. K1535) 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® D1 CHO-K1 DA cells and GeneBLAzer® D1-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC_{50} concentrations of dihydrexidine (Figure 1). In addition, GeneBLAzer® D1-CRE-*bla* CHO-K1 cells have been tested for assay performance under variable conditions. Additional testing data using alternate stimuli are also included.

Target Description

Receptors for the neurotransmitter dopamine in the brain have long been targets for anti-psychotic and Parkinson's disease drugs (1-4). The dopamine family of GPCR's consists of 5 members that are separated into two groups based on sequence homology: D1-like and D2-like. The D1-like receptors are D1 and D5 (5, 6), while D2, D3 (7), and D4 (8) make up the D2-like group. Activaton of the D1-like receptors (D1 and D5) is associated with activation of adenylate cyclase whereas activation of D2-like receptors (D2, D3, and D4) is associated with inhibition of adenylate cyclase activity.

Validation Summary

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

1. Dihydrexidine agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	71 nM	77 nM
Z'-factor	0.92	0.89

Recommended cell no. = 10K cells/well
Recommended [DMSO] = 0.1-1%
Recommended Stim. Time = 5 hours
Max. [Stimulation] = 35 µM

2. Alternate agonist dose response

SKF38393 EC_{50} = 22 nM
Fenoldopam EC_{50} = 32 nM
SKF75670 EC_{50} = 86 nM
Dopamine EC_{50} = 454 nM

3. Antagonist Dose Response

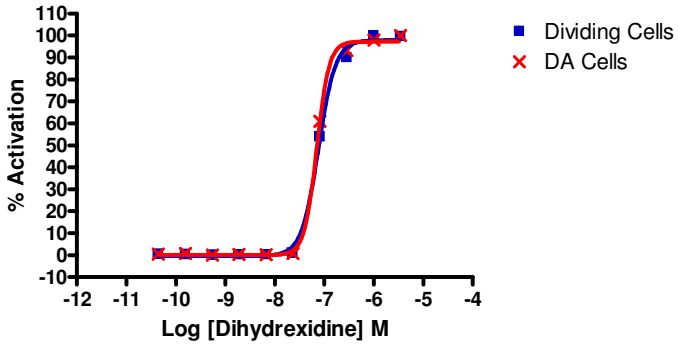
SCH23390 IC_{50} = 42 nM
SKF-83566 IC_{50} = 89 nM

4. Agonist 2nd Messenger Response

Dihydrexidine EC_{50} = 47 nM

Primary Agonist Dose Response

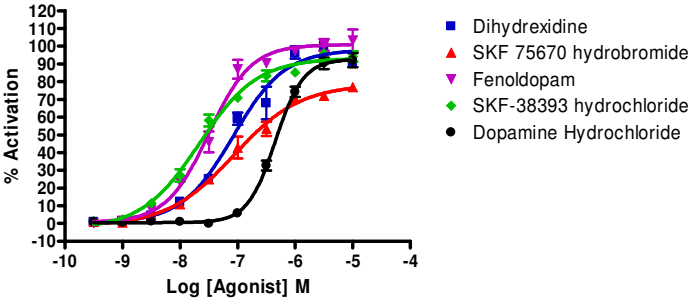
Figure 1 — GeneBLAzer® D1 CHO-K1 DA and D1-CRE-*bla* CHO-K1 dose response to dihydrexidine under optimized conditions



GeneBLAzer® D1 CHO-K1 DA cells and GeneBLAzer® D1-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 dihydrexidine (Phoenix Pharmaceutical #019-06) 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 dihydrexidine (n=6 for each data point).

Alternate Agonist Dose Response

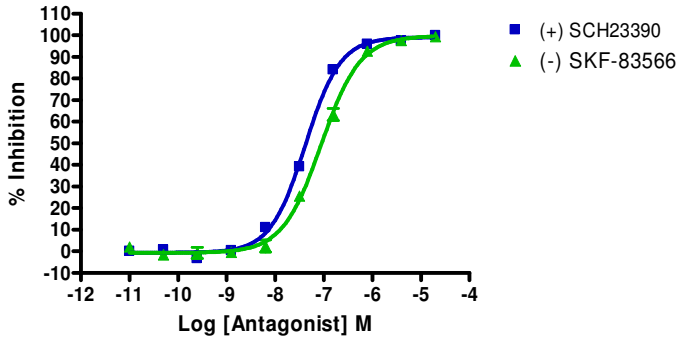
Figure 2 — GeneBLAzer® D1-CRE-*bla* CHO-K1 dose response to alternate agonists under optimized conditions



GeneBLAzer® D1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before the assay in a 384-well format and stimulated with Dihydrexidine, SKF-75670, Fenoldopam, SKF-38393, or Dopamine over the indicated concentration range 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 the % Activation plotted against the indicated concentrations of agonist (n=2 for each data point).

Antagonist Dose Response

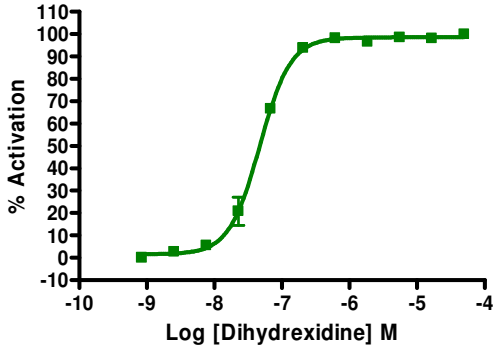
Figure 3— GeneBLAzer® D1-CRE-*bla* CHO-k1 antagonist dose response under optimized conditions



GeneBLAzer® D1-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated in a 384-well assay plate and incubated for 16-20 hours. Cells were then incubated with a dilution series of antagonist for 30 min. at 37°C followed by a 5 hour incubation with an EC_{80} concentration of dihydrexidine (Phoenix Pharmaceutical #019-06) in 0.1% DMSO. 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 antagonist.

Agonist 2nd Messenger Dose Response

Figure 4— GeneBLAzer® D1-CRE-*bla* CHO-k1 2nd messenger dose response to dihydrexidine under optimized conditions.



GeneBLAzer® D1-CRE-*bla* CHO-K1 cells were tested for a response to dihydrexidine (Phoenix Pharmaceutical #019-06) with a TR-FRET cAMP assay

References

1. Carlsson, A., Lindqvist, M. and Magnusson, M. (1957) **3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists.** *Nature*, **180**, 1200.

2. Carlsson, A., Lindqvist, M., Magnusson, T. and Walbeck, B. (1958) **On the presence of 3-hydroxytyramine in brain.** *Science*, **127**, 471 .

3. Carlsson, A. and Lindqvist, M. (1963) **Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain**. *Acta Pharmacol. Toxicol.*, **20**, 140 - 144.

4. Moran, T. H., Robinson, P. H., Goldrich, M. S. and McHugh, P. R. (1963) **Die topishe Lokalisation und das Verhalten von Noradrenalin und Dopamin (3-hydroxytyramin) in der Substantia nigra des normalen und Parkinson-kranken Menschen.** *Wien. Klin. Wochenschr.*, **75**, 309 - 312.

5. Van Tol, H. H. M. et al. (1992) **Multiple dopamine D4receptor variants in the human population**. *Nature*, **358**, 149 - 152.

6. Sunahara, R. K. et al. (1991) **Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1.** *Nature*, **350**, 614 - 619.

7. Sokoloff, P., Giros, B., Martres, M.-P., Bouthenet, M.-L. and Schwartz, J.-C. (1990) **Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics.** *Nature*, **347**, 146 - 151.

8. Van Tol, H. H. M., Bunzow, J. R., Guan, H. C., Sunahara, R. K., Seeman, P., Niznik, H. B. and Civelli, O. (1991) **Cloning of the gene for a human dopamine D4receptor with high affinity for the antipsychotic clozapine.** *Nature*, **350**, 610 - 614.