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**Optimization of the GeneBLAzer® HTR7-CRE-*bla* CHO-K1 Cell Line**

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**GeneBLAzer® HTR7 CHO-K1 DA Assay Kit****GeneBLAzer® HTR7 CRE-*bla* CHO-K1 Cells**

Catalog Numbers – K1301 and K1704

**Cell Line Descriptions**

GeneBLAzer® HTR7 CHO-K1 DA (Division Arrested) cells and GeneBLAzer® HTR7-CRE-*bla* CHO-K1 cells contain the human 5-hydroxytryptamine (serotonin) receptor 7 (Accession# [NM\\_019860.2](#)) 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® HTR7 CHO-K1 DA cells and GeneBLAzer® HTR7-CRE-*bla* CHO-K1 cells are functionally validated for Z'-factor and EC<sub>50</sub> concentrations of 5-hydroxytryptamine (5-HT); (Figure 1). In addition, GeneBLAzer® HTR7-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**

Drugs targeting serotonin receptors have shown promise in the treatment of numerous diseases or conditions, including neurological disorders (schizophrenia, anxiety, cognitive dysfunction, bipolar affective disorder), migraine headaches, obesity, irritable bowel syndrome, and chemotherapy-induced nausea and vomiting. In particular, the 5-HT<sub>7</sub> receptor (HTR7) is considered to be a possible schizophrenia-susceptibility factor, and has been implicated in human anxiety. Additional studies linking the 5-HT<sub>7</sub> receptor to bipolar affective disorder have been inconclusive.

## Validation Summary

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

### 1. 5-HT agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC <sub>50</sub>	70 nM	68 nM
Z'-factor	0.85	0.85

Recommended cell no.	= 10K cells/well
Recommended [DMSO]	= 0.1-1%
Recommended Stim. Time	= 4 hours
Max. [Stimulation]	= 3.3 μM

### 2. Antagonist Dose Response

SB269970 IC <sub>50</sub>	= 889 pM
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### 3. Agonist 2<sup>nd</sup> Messenger Dose Response

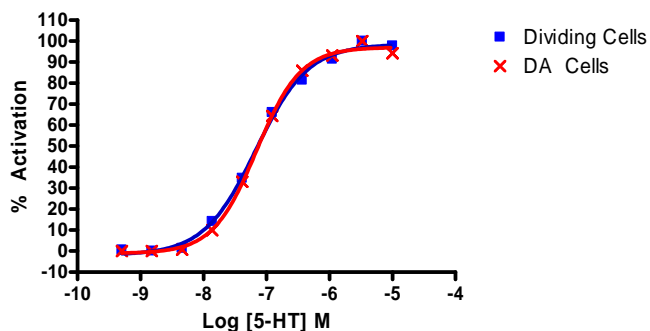
5-HT EC <sub>50</sub>	= 2.7 nM
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## Assay Testing Summary

- Assay performance with variable cell number
- Assay performance with variable stimulation time
- Assay performance with variable substrate loading time
- Assay performance with variable DMSO concentration

## Primary Agonist Dose Response

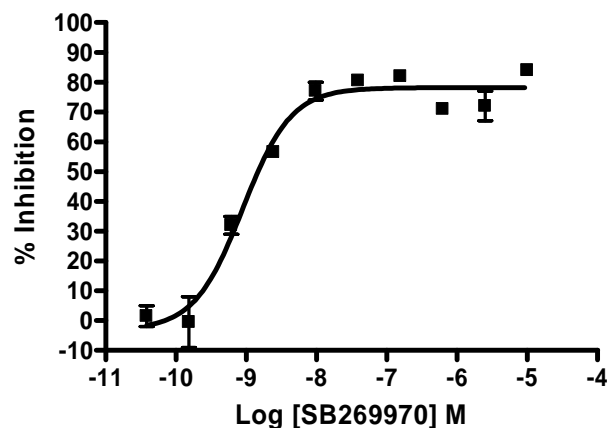
**Figure 1 — GeneBLAzer® HTR7 CHO-K1 DA and HTR7-CRE-*bla* CHO-K1 dose response to 5-HT under optimized conditions**



GeneBLAzer® HTR7 CHO-K1 DA cells and HTR7-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-HT 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 for each replicate against the concentrations of 5-HT (n=6 for each data point).

## Antagonist Dose Response

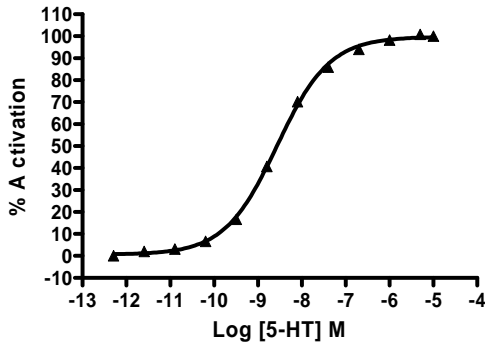
**Figure 2 — GeneBLAzer® HTR7-CRE-*bla* CHO-K1 dose response to SB269970 under optimized conditions**



GeneBLAzer® HTR7-CRE-*bla* CHO-K1 cells (5,000 cells/well) were plated in a 384-well format and incubated for 16-24 hours. Cells were then treated with SB269970 over the indicated concentration range in the presence of 0.1% DMSO for 30 minutes prior to stimulation with an EC<sub>80</sub> concentration of 5-HT. 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 % Inhibition plotted against the indicated concentrations of serotonin antagonist (n=2 for each data point).

## Agonist 2<sup>nd</sup> Messenger Dose Response

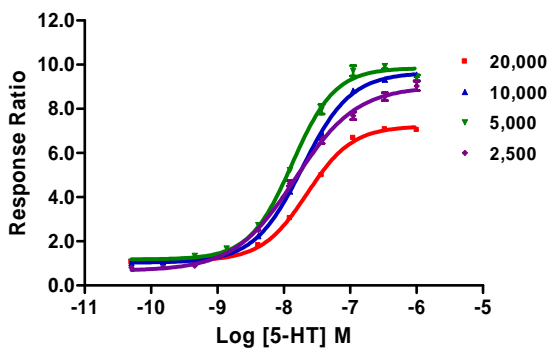
Figure 3— GeneBLazer® HTR7-CRE-*bla* CHO-k1 2<sup>nd</sup> messenger dose response to 5-HT under optimized conditions



GeneBLazer® HTR7-CRE-*bla* CHO-K1 cells were tested for a response to 5-HT with a TR-FRET cAMP assay (n=4 for each data point).

## Assay Performance with Variable Cell Number

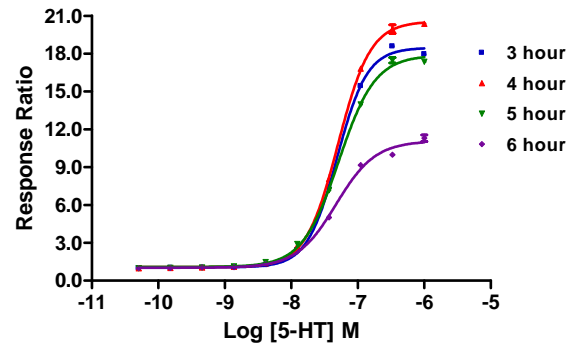
Figure 4 — GeneBLazer® HTR7-CRE-*bla* CHO-K1 dose response to 5-HT with 2.5, 5, 10, and 20K cells/well



GeneBLazer® HTR7-CRE-*bla* CHO-K1 cells were plated the day before agonist addition at 2,500, 5,000, 10,000, or 20,000 cells/well in a 384-well format. Cells were stimulated with 5-HT (Sigma #H-9523) 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 460/530 ratios plotted for each cell number against the indicated concentrations of 5-HT (n=8 for each data point).

## Assay Performance with Variable Stimulation Time

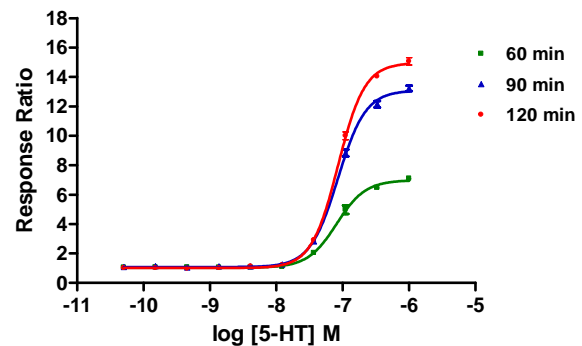
Figure 5 – GeneBLazer® HTR7-CRE-*bla* CHO-K1 dose response to 5-HT with 3, 4, 5, and 6 hour stimulation times



GeneBLazer® HTR7-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before agonist addition in a 384-well assay plate. 5-HT (Sigma #H-9523) was then added to the plate over the indicated concentration range. Plates were stimulated for 3, 4, 5, or 6 hrs with 5-HT 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 for each stimulation time against the indicated concentrations of CRF (n=8 for each data point).

## Assay Performance with Variable Substrate Loading Time

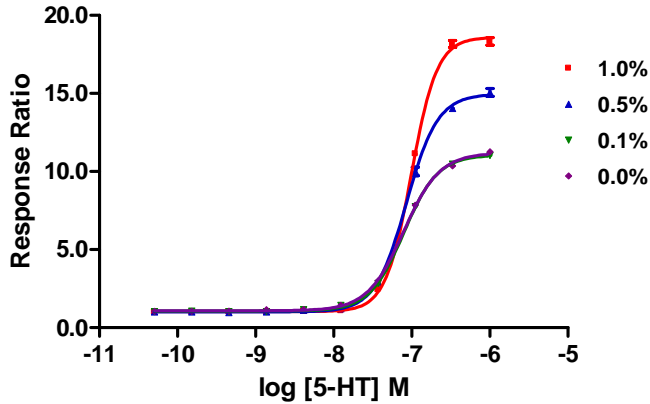
Figure 6 — GeneBLazer® HTR7-CRE-*bla* CHO-K1 dose response to 5-HT with 60, 90 and 120 minute substrate loading times



GeneBLazer® HTR7-CRE-*bla* CHO-K1 cells were plated the day of the assay at 10,000 cells/well in a 384-well format. Cells were stimulated with 5-HT (Sigma #H-9523) over the indicated concentration range in the presence of 0.5% DMSO for 4 hours. Cells were then loaded with LiveBLazer™-FRET B/G Substrate (2µM final concentration of CCF4-AM + 1mM Solution D) for 60, 90, or 120 minutes. Fluorescence emission values at 460 nm and 530 nm for the various substrate loading times were obtained using a standard fluorescence plate reader and the Response Ratios plotted for each substrate loading time against the indicated concentrations of 5-HT (n=16 for each data point).

## Assay Performance with Variable DMSO Concentration

Figure 7 – GeneBLAzer® HTR7-CRE-*bla* CHO-K1 dose response to 5-HT with 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® HTR7-CRE-*bla* CHO-K1 cells (10,000 cells/well) were plated the day before agonist addition in a 384-well black-walled tissue culture assay plate. DMSO was then added to the assay at concentrations from 0% to 1%, and 5-HT (Sigma #H-9523) was added to the plate over the indicated concentration range. Plates were stimulated for 4 hrs 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 for each DMSO concentration were plotted against the indicated concentrations of 5-HT (n=8 for each data point).