GeneBLAzer® H1 HEK 293T DA Cells

GeneBLAzer® H1 NFAT-*bla* HEK 293T Cells

Catalog Numbers – K1299 and K1703

Cell Line Descriptions

GeneBLAzer® H1 HEK 293T DA (Division Arrested) cells and GeneBLAzer® H1-NFAT-*bla* HEK 293T cells contain the human Histamine Subtype 1 receptor (H1), (Accession # NM_000861) stably integrated into the CellSensor® NFAT-*bla* HEK 293T cell line. CellSensor® NFAT-*bla* HEK 293T cells (Cat. no. K1538) contain a beta-lactamase (*bla*) reporter gene under control of the NFAT response element. 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® H1 HEK 293T DA cells and GeneBLAzer® H1-NFAT-*bla* HEK 293T cells are functionally validated for Z'-factor and EC_{50} concentrations of Histamine (Figure 1). In addition, GeneBLAzer® H1-NFAT-*bla* HEK 293T 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

Histamine is synthesized in a restricted population of neurons (1) and stored mainly in mast cells, basophils and enterochromaffin cells. During an allergic reaction, Histamine is released from these cells and leads to the classical symptoms of the skin and airway (2). It was thought, until the seventies, that there was only one Histamine receptor. During development of Pyrilamine, it was discovered that this antihistamine did not antagonize cells of the stomach and the heart (3-4). This discovery led to the identification of the H2 receptor (4), which regulates gastric acid secretion. More recently, it was discovered that Histamine also regulates the release of several important neurotransmitters (e.g. dopamine and serotonin). These findings led to the discovery of the third histamine (H3) receptor, which has little homology to H1 and H2 receptors (5-6). Using the H3 sequence as a template, the fourth Histamine (H4) receptor was discovered. The histamine H4 receptor is involved in the chemotaxis of leukocytes and mast cells to sites of inflammation and is suggested to be a potential drug target for asthma and allergy (7-8).

H1 receptors are present in many tissues throughout the body and play an important role in several pathophysiological conditions, including allergic responses in the brain, lungs, heart and smooth muscles throughout the cardiovascular system. In addition, the H1 receptor is also present in the kidneys, skeletal muscle and lymphocytes (7-8).

Validation Summary

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

1. Histamine agonist dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	29 nM	33 nM
Z'-Factor	0.88	0.85

Optimum cell no.	= 5K cells/well
Optimum[DMSO]	= up to 0.5%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 6.4µM

2. Alternate agonist dose response

HTMT EC_{50}	= 7µM

3. Antagonist dose response

Tripolidine IC_{50}	= 98 pM
Pyrilamine IC_{50}	= 4.7 nM
Chloropheniramine IC_{50}	= 9.2 nM

4. Agonist 2nd messenger response

Histamine EC_{50}	= 22 nM

Assay Testing Summary

5. Assay performance with variable cell number

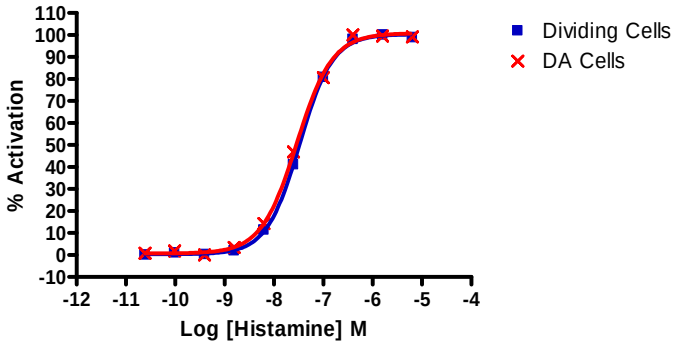
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

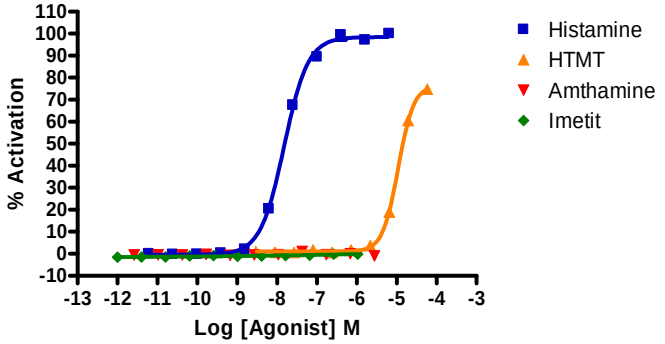
Figure 1 — GeneBLAzer® H1 HEK 293T DA and GeneBLAzer® H1-NFAT-*bla* HEK 293T dose response to Histamine under optimized conditions



GeneBLAzer® H1 HEK 293T DA cells and GeneBLAzer® H1-NFAT-bla HEK 293T 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 Histamine 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 Histamine (n=6 for each data point).

Alternate Agonist Dose Response

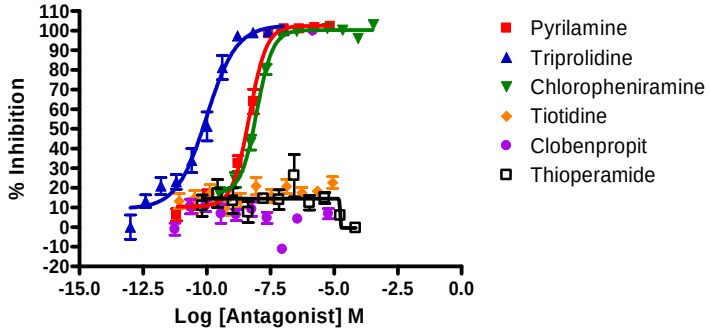
Figure 2 — GeneBLAzer® H1-NFAT-*bla* HEK 293T dose response to Histamine, HTMT, Amthamine and Imetit



GeneBLAzer® H1-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated 16-20 hours prior to assay in a 384-well format. On the day of the assay, cells were stimulated with dilution series of Histamine (Sigma #H7250), HTMT (Tocris #0646), Amthamine (Tocris #0668), and Imetit (Sigma #I135) 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 is shown plotted against the concentrations of Histamine, HTMT, Amthamine, and Imetit (n=8 for each data point). The data shows the correct rank order potency for Histamine and HTMT and selectivity for H1 receptor agonists. Amthamine is an H2 selective agonist, and Imetit is an H3/H4 agonist.

Antagonist Dose Response

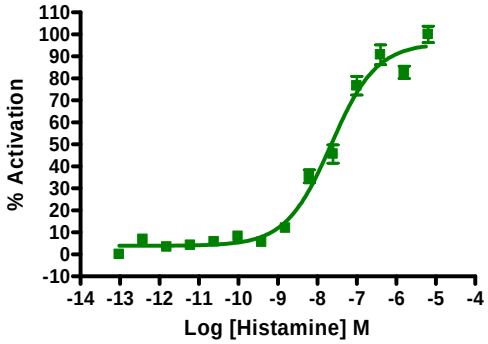
Figure 3 — GeneBLAzer® H1-NFAT-*bla* HEK 293T dose response to Pyrilamine, Triprolidine, Chlorpherniramine, Tiotidine, Clobenpropit and Thioperamide



GeneBLAzer® H1-NFAT-*bla* HEK 293T cells were plated 16-20 hours prior to assay at 5,000 cells per well in a 384-well format. Dilitions series of Pyralimine (Sigma #P5514), Triprolidine (Sigma #T6764), Tiotidine (Tocris #0825), Chloropheniramine (Sigma #C4915), ,Clobenpropit (Sigma #C209), and Thioperamide (Sigma #T123) in the presence of 0.25% DMSO. Cells were incubated at 37ºC & 5% CO_2 for 30 min. Histamine (Sigma #H7250) was thenadded to the plate at the EC_{80} concentration of 66.5 nM along with 0.25% DMSO (0.5% Final concentration) Cells were incubated for 5 hours and 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 concentrations of the antagonists. The data shows the correct rank order potency for Pyrilamine, Triprolidine and Chloropheniramine (each are H1 selective antagonists), and selectivity Clobenpropit is an H3/H4 selective antagonist, Thiperamide is an H2 antagonist, and Tiotidine is an H2 antagonist (n=8 for each data point).

Agonist 2nd Messenger Response

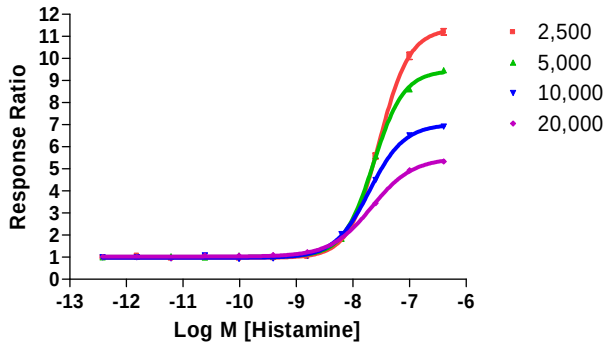
Figure 4 — GeneBLAzer® H1-NFAT-*bla* HEK 293T 2nd messenger dose response to Histamine under optimized conditions



GeneBLAzer® H1-NFAT-*bla* HEK293T cells were loaded with Fluo4-AM and tested for a response to Histamine.

Assay Performance with Variable Cell Number

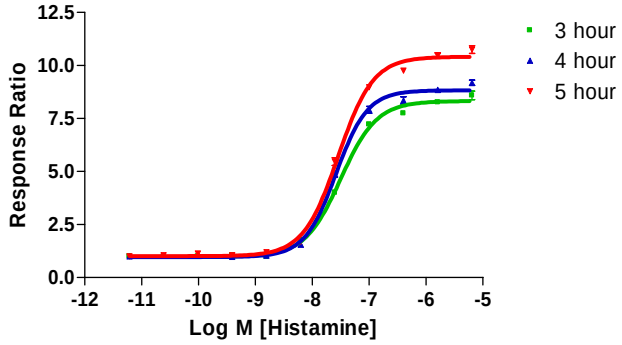
Figure 5 – GeneBLAzer® H1-NFAT-*bla* HEK 293T dose response using 2.5, 5, 10, and 20K cells/well



GeneBLAzer® H1-NFAT-*bla* HEK 293T cells were plated 16-20 hours prior to assay at 2,500 5,000 10,000 or 20,000 cells/well in a 384-well format. On the day of the assay, cells were stimulated with a dilution series of Histamine (Sigma #H7250) 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 plotted for each cell number against the concentrations of Histamine (n=8 for each data point).

Assay Performance with Variable Stimulation Time

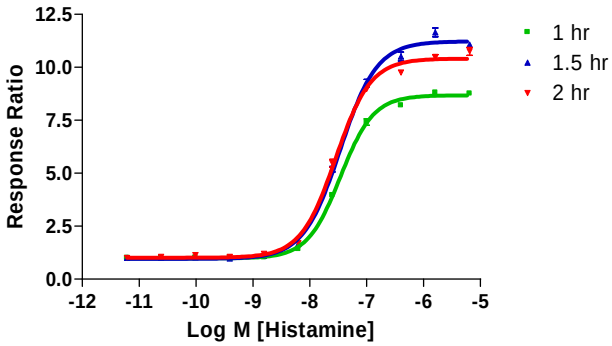
Figure 6 – GeneBLAzer® H1-NFAT-*bla* HEK 293T dose response using 3, 4 and 5 hr stimulation times



GeneBLAzer® H1-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated 16-20 hours prior to assay in a 384-well format. On the day of the assay, cells were stimulated with a dilution series of Histamine (Sigma #H7250) 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 fluorescence plate reader and the Response Ratios plotted for each stimulation time against the concentrations of Histamine (n=8 for each data point).

Assay Performance with Variable Substrate Loading Times

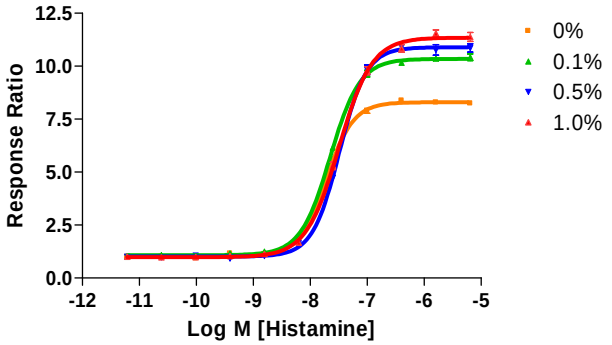
Figure 7 – GeneBLAzer® H1-NFAT-*bla* HEK 293T dose response using 1, 1.5, and 2 hour substrate loading times.



GeneBLAzer® H1-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated 16-20 hours prior to assay in a 384-well format. On the day of assay, cells were stimulated with a dilution series of Histamine (Sigma #7250) 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 fluorescence plate reader and the Response Ratios plotted for each substrate loading time against the concentrations of Histamine (n=8 for each data point).

Assay Performance with Variable DMSO Concentration

Figure 8 – GeneBLAzer® H1-NFAT-*bla* HEK 293T dose response using 0, 0.1, 0.5 and 1% DMSO



GeneBLAzer® H1-NFAT-*bla* HEK 293T cells (5,000 cells/well) were plated 16-20 hours prior to assay in a 384-well format. Cells were stimulated with a dilution series of Histamine (Sigma #H7250) 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 fluorescence plate reader and the Response Ratios plotted for each DMSO concentration against the concentrations of Histamine(n=8 for each data point).

References

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