GeneBLAzer® H2 HEK 293T DA Cells

GeneBLAzer® H2 CRE-*bla* HEK 293T Cells

Catalog Numbers – K1307 and K1707

Cell Line Descriptions

GeneBLAzer® H2 HEK 293T DA (Division Arrested) cells and GeneBLAzer® H2-CRE-*bla* HEK 293T cells contain the human Histamine Subtype 2 receptor (H2), (Accession #NM_022304) stably integrated into the CellSensor® CRE-*bla* HEK 293T cell line. CellSensor® CRE-*bla* HEK 293T cells (Cat. no. K1540) 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® H2 HEK 293T DA cells and GeneBLAzer® H2-CRE-*bla* HEK 293T cells are functionally validated for Z'-factor and EC_{50} concentrations of Histamine (Figure 1). In addition, H2-CRE-*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).

H_2 receptors located in the stomach and gastric parietal cells regulate gastric acid secretion, whereas those located in smooth muscles (airway, vascular and uterine) are involved in relaxation. Lymphocyte function is inhibited by the H_2 receptors. H2 receptors are also located in the brain, heart, neutrophils, basophils and mast cells (7, 8). Histamine is the endogenous agonist of the H2 pathway.

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}	15 nM	17 nM
Z'-factor	0.68	0.87

Optimum cell no.	= 10K cells/well
Optimum [DMSO]	= up to 1%
Optimum Stim. Time	= 5 hours
Max. [Stimulation]	= 33nM

2. Alternate agonist dose response

Amthamine	EC_{50}	= 9 nM
Dimaprit	EC_{50}	= 38 nM

3. Antagonist dose response

Tiotidine	IC_{50}	= 30 nM
Rantadine	IC_{50}	= 433 nM

4. 2nd messenger dose response

Histamine EC_{50} = 2.7 nM

Assay Testing Summary

5. Assay performance with variable cell number

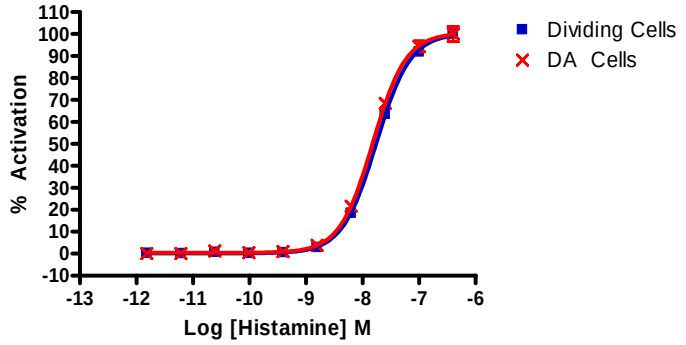
6. Assay performance with variable stimulation time

7. Assay performance with variable substrate loading time

8. Assay performance with variable DMSO concentration

Primary Agonist Dose Response

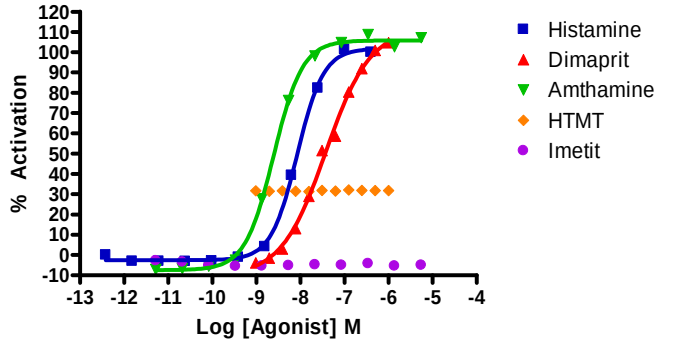
Figure 1 — GeneBLAzer® H2 HEK 293T DA and GeneBLAzer® H2-CRE-*bla* HEK 293T dose response to Histamine under optimized conditions



GeneBLAzer® H2 HEK 293T DA cells and GeneBLAzer® H2-CRE-*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 % Activation plotted for each replicate against the concentrations of Histamine (n=6 for each data point).

Alternate Agonist Dose Response

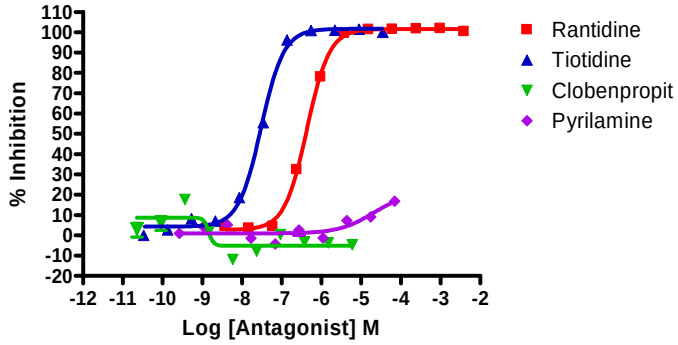
Figure 2 — GeneBLAzer® H2-CRE-*bla* HEK 293T dose response to Histamine, Dimaprit, Amthamine, HTMT and Imetit



GeneBLAzer® H2-CRE-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well format. Cells were stimulated with a dilution series of Histamine (Sigma H7250) HTMT (Tocris 0646), Amthamine (Tocris 0668), or Imetit (Sigma I135) 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 is shown plotted against the concentrations of agonist (n=8 for each data point). The data shows the correct rank order potency and selectivity for Histamine, Dimaprit, and Amthamine. HTMT and Imetit are H1 and H3/H4 agonists, respectively.

Antagonist Dose Response

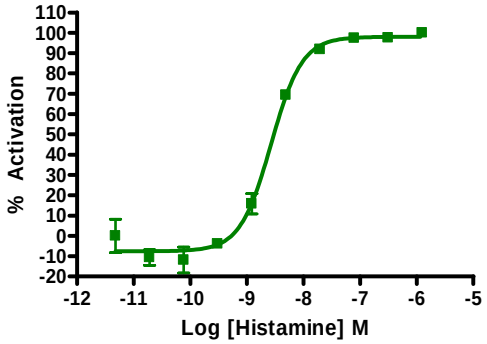
Figure 3 — GeneBLAzer® H2-CRE-*bla* HEK 293T dose response to Rantidine, Tiotidine, Clobenpropit, and Pyrilamine



GeneBLAzer® H2-CRE-*bla* HEK 293T cells (10,000 cells/well) were plated the day of the assay in a 384-well assay plate. Cells were treated with either Rantidine (Sigma R101), Tiotidine (Tocris 0825), Clobenpropit (Sigma C209) or Pyrilamine (Sigma P5514) in 0.25% DMSO and then incuvated at 37°C/ 5%CO2 for 30 min. Histamine (33nM) was added to the plate at EC80 concentration of 33nM 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 (n=8 for each data point). The data shows the correct rank order potency and selectivity for Rantidine and Tiotidine. Clobenpropit and Pyrilamine are H3/H4 and H1 receptor antagonists, respectively.

2nd messenger dose response

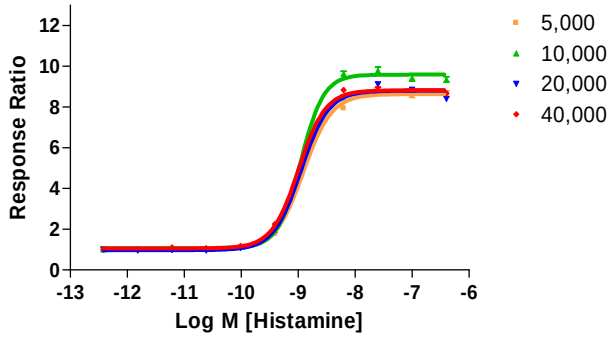
Figure 4 — GeneBLAzer® H2-CRE-*bla* HEK 293T 2nd messenger dose response to Histamine



GeneBLAzer® H2-CRE-*bla* HEK293T cells were tested for a response to histamine with a TR-FRET cAMP assay.

Assay Performance with Variable Cell Number

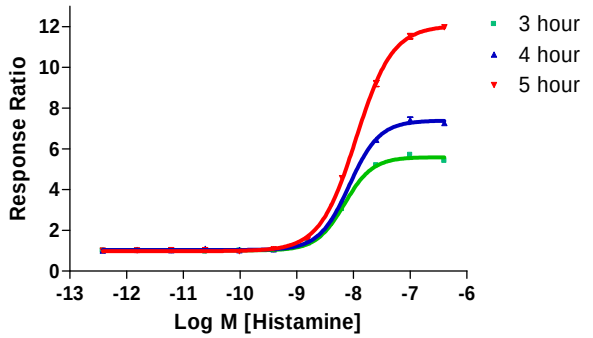
Figure 5 – GeneBLAzer® H2-CRE-*bla* HEK 293T dose response using 5, 10, 20 and 40K cells / well



GeneBLAzer® H2-CRE-*bla* HEK 293T cells were plated on the day of the assay at 5,000 10,000 or 20,000 and 40,000 cells/well in a 384-well format. 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 for 2 hours with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission values at 460 nm and 530 nm for the various cell numbers were obtained using a standard fluorescence plate reader 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

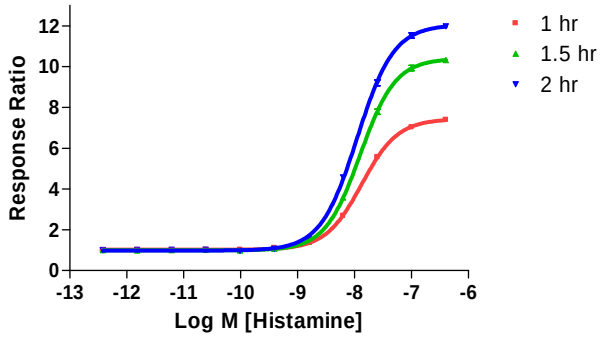
Figure 6 – GeneBLAzer® H2-CRE-*bla* HEK 293T dose response using 3, 4, and 5 hr stimulation time



GeneBLAzer® H2-CRE-*bla* HEK 293T cells were plated the day of the assay at 10,000 cells/well in a 384-well format. Cells were stimulated for 3, 4 or 5 hours with a dilution series of Histamine (Sigma H7250) in the presence of 0.5% DMSO. Cells were thenloaded 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 Time

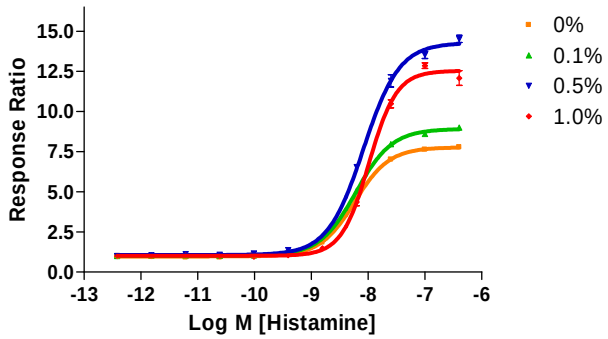
Figure 7 – GeneBLAzer® H2-CRE-*bla* HEK 293T dose response using 1, 1.5, and 2 hr load time



GeneBLAzer® H2-CRE-*bla* HEK 293T cells were plated at 10,000 cells/well the day of the assay in a 384-well format. Cells were stimulated for 5 hours with a dilution series of Histamine (Sigma H7250) in the presence of 0.5% DMSO. 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® H2-CRE-*bla* HEK 293T dose response using 0, 0.1, 0.5, and 1% DMSO



GeneBLAzer® H2-CRE-*bla* HEK 293T cells were plated the day of the assay at 10,000 cells/well in a 384-well format. Cells were stimulated for 5 hours with a dilution series of Histamine (Sigma H7250). DMSO was added to the assay 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 are shown plotted for each DMSO concentration against the concentrations of Histamine (n=8 for each data point).

References

1. Schwartz, J.C. *et al*. **Histaminergic transmission in the mammalian brain**. *Physiol. Revs*. **71**, 1-51 (1991)

2. Ring et al. **Histamine and allergic diseases**.*New Trends in Allergy*. Pp 44 (1985).

3. Black, *et al*. **Definition and antagonism of histamine H2 receptors**. *Nature* **236**, 385. (1972)

4. Ash and Schild. **Receptors mediating some actions of histamine**. *British Journal of Pharmacology* ***27***, 427.

5. Lovenberg, T.W. *et al*. **Cloning and functional expression of the human histamine H3 receptor**. *Mol. Pharmacol*. **55**, 1101-1107 (1999).

6. Moriset, S. *et al*. **High constitutive activity of native H3 receptors regulate histamine neurons in the brain**. *Nature* **408**, 860-864 (2000).

7. Hill, S.J. *et al*. **Histamine receptors**. *The IUPHAR Compendium of Receptor Characterization and Classification, 2nd edition*, pp. 227-232, IUPHAR Media, London, UK (2000).

8. Hill, S.J. *et al*. **International Union of Pharmacology. XIII. Classification of Histamine Receptors** *Pharmacol.Rev*. **49**,253-278 (1997).