Tango™ H3-*bla* U2OS DA Assay Kit

Tango™ H3-*bla* U2OS cells

Catalog Numbers – K1509, K1466

Cell Line Descriptions

Tango™ H3-*bla* U2OS DA (Division Arrested) cells and Tango™ H3-*bla* U2OS cells contain the human Histamine Receptor H3 (H3) linked to a TEV protease site and a Gal4-VP16 transcription factor stably integrated into the Tango™ GPCR-*bla* U2OS parental cell line. This parental cell line stably expresses a beta-arrestin/TEV protease fusion protein and the beta-lactamase (*bla*) reporter gene under the control of a UAS response element. Division Arrested (DA) cells are available in 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 the Tango™ H3-*bla* U2OS cells and the Tango™ H3-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of Methylhistamine (Figure 1). In addition, Tango™ H3-*bla* U2OS cells have been tested for assay performance under variable conditions.

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_3 receptors are found in the following areas of the central and peripheral nervouse systems: thalamus, caudate nucleus, putamen, cerebellum, amygdala, substantia nigra, hippocampus, hypothalamus and cerebral cortex (9). These receptors have been reported to be involved in vasoconstriction, regulation of food intake, body weight, and inhibition of chemical and mechanical nociception (10-12).

Validation Summary

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

1. Methylhistamine dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	57.8 nM	57.6 nM
Z'-factor	0.66	0.78

Recommended cell no. /well = 10,000
Recommended Stim. Time = 16 hrs
Max. [Stimulation] = 50,000 nM

2. Alternate agonist dose response

Histamine EC_{50} = 423 nM

3. Antagonist dose response

Thioperamide IC_{50} = 246 nM

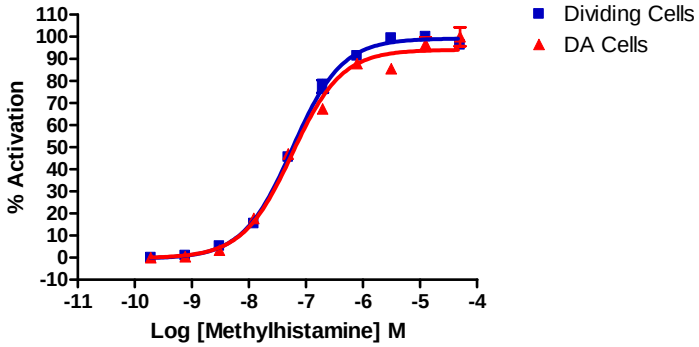
Assay Testing Summary

4. Assay performance with variable stimulation time.

See graph below

Primary Agonist Dose Response

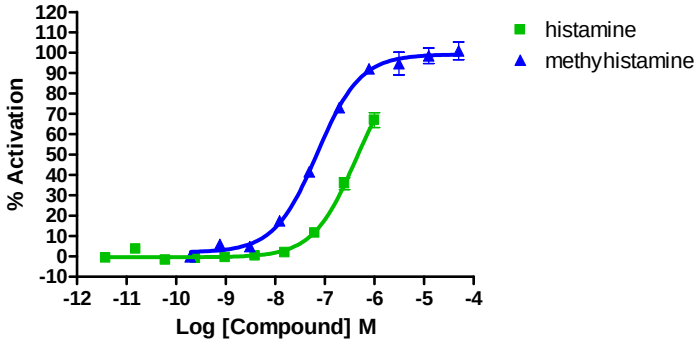
Figure 1 — Tango™ H3-*bla* U2OS cells and Tango™ H3-*bla* U2OS DA cells dose response to Methylhistamine under optimized conditions



Tango™ H3-*bla* U2OS cells and Tango™ H3-*bla* U2OS DA 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 Methylhistamine (Tocris 569) 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 % Activation plotted for each replicate against the concentrations of Methylhistamine.

Alternate Agonist Dose Response

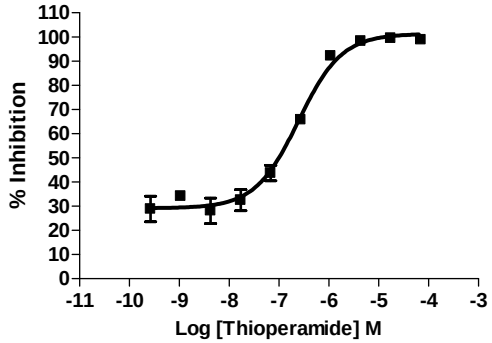
Figure 2 — Tango™ H3-*bla* U2OS cells dose response to Histamine and Methylhistamine.



Tango™ H3-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with Methylhistamine (Tocris 569), or Histamine (Sigma H7250) 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. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the 460/530 Ratios plotted against the indicated concentrations of agonist. The data shows the correct rank order potency.

Antagonist Dose Response

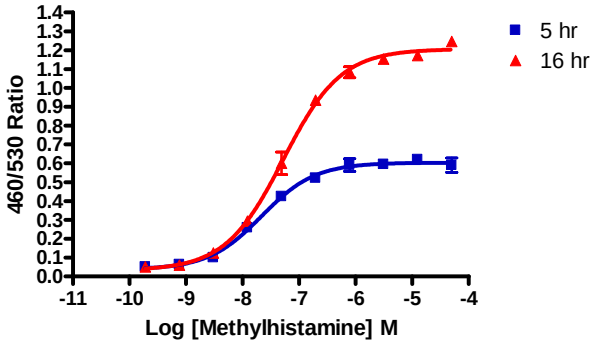
Figure 3 — Tango™ H3-*bla* U2OS cells dose response to Thioperamide



Tango™ H3-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to Thioperamide (Sigma T123) for 30 min. and then stimulated with an EC80 concentration of Methylhistamine (Tocris 569) 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of Thioperamide.

Assay Performance with Variable Stimulation Time

Figure 4 – Tango™ H3-*bla* U2OS cells dose response to Methylhistamine with 5 or 16 hour stimulation times



H3-*bla* U2OS cells (10,000 cells/well) were plated at 10,000 cells/well in a 384 well plate and incubated for 16-24 hours. Methylhistamine (Tocris 569) in 0.1% DMSO was either added at the time of plating (for the 16 hour assay) or was added for 5 hours after the overnight incubation (for the 5 hour assay). The 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 460/530 Ratio plotted for each replicate against the concentrations of Methylhistamine.

References

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