Tango™ H4-*bla* U2OS cells

Catalog Numbers – K1465

Cell Line Descriptions

Tango™ H4-*bla* U2OS cells contain the human Histamine Receptor H4 (H4) 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.

The Tango™ H4-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of 4-Methylhistamine (Figure 1). In addition, Tango™ H4-*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 second histamine receptor (HRH2) (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 (HRH3) receptor, which has little homology to HRH1 and HRH2 receptors (5,6). Using the HRH3 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).

Validation Summary

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

1. **4-Methylhistamine dose response under optimized conditions**

   Dividing Cells

EC_{50}	= 99.5 nM
Z'-factor	= 0.58
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 16 Hrs.
Max. [Stimulation]	= 40000 nM

2. **Alternate agonist dose response**

   Histamine EC_{50} = 132 pM

3. **Antagonist dose response**

   Thioperamide IC_{50} = 154 nM

4. **Agonist 2nd messenger response**

   4-Methylhistamine EC_{50} = 27 nM

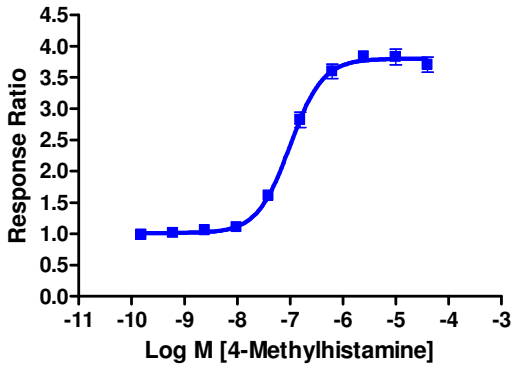
Assay Testing Summary

5. **Assay performance with variable stimulation times.**

6. **Assay performance with variable serum starve times.**

Primary Agonist Dose Response

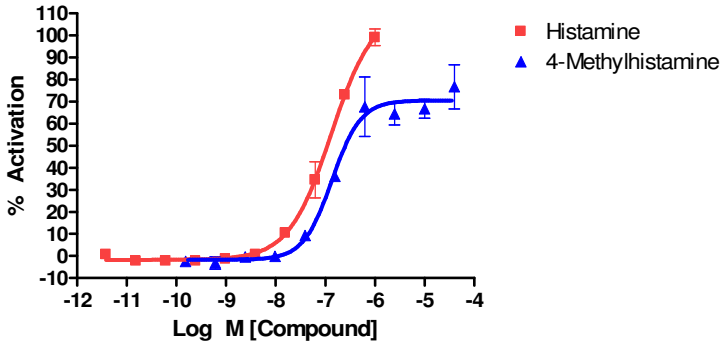
Figure 1 — Tango™ H4-*bla* U2OS cells and Tango™ H4-*bla* U2OS DA cells dose response to 4-Methylhistamine under optimized conditions



Tango™ H4-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 44-48 hours. Cells were stimulated with a dilution series of 4-Methylhistamine (Tocris 2342) in the presence of 0.1% DMSO for 16 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 4-Methylhistamine.

Alternate Agonist Dose Response

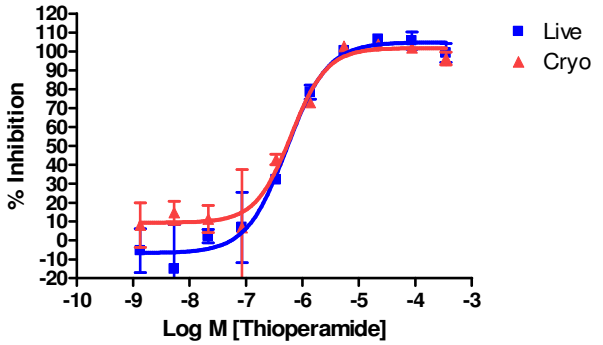
Figure 2 — Tango™ H4-*bla* U2OS cells dose response to Histamine and 4-Methylhistamine.



Tango™ H4-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with Histamine (Sigma H7250), and 4-Methylhistamine (Tocris, 2342) 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 % Activation plotted against the indicated concentrations of agonist. The data shows the correct rank order potency.

Antagonist Dose Response

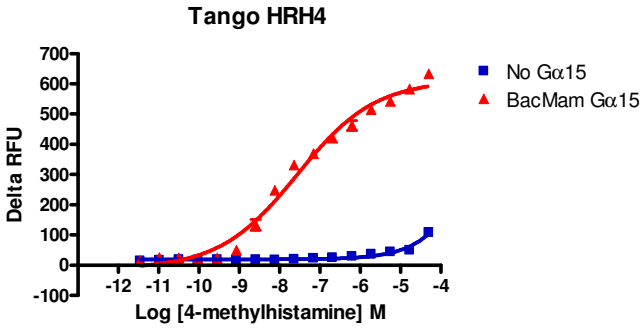
Figure 3 — Tango™ H4-*bla* U2OS cells dose response to Thioperamide



Tango™ H4-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 44-48 hours. Cells were exposed to Thioperamide (Sigma T123) for 30 min. and then stimulated with an EC80 concentration of 4-Methylhistamine (Tocris 2342) in the presence of 0.1% DMSO for 16 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.

Agonist 2nd Messenger Response

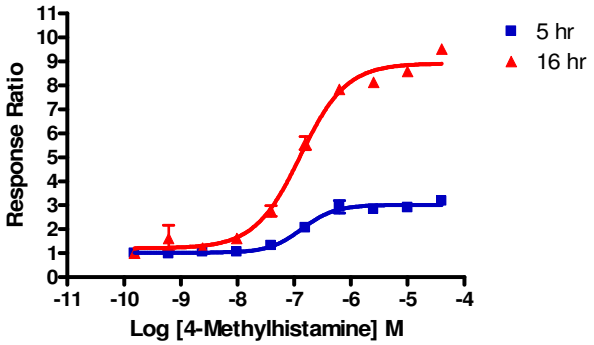
Figure 4 — Tango™ H4-*bla* U2OS cells transduced with a BacMam Gα15 construct 2nd messenger dose response to 4-Methylhistamine



Tango™ H4-*bla* U2OS cells were transduced overnight with a BacMam Gα15 construct or left untransduced. Cells were then loaded with Fluo-4 Direct™ and tested for a response to 4-methylhistamine.

Assay Performance with Variable Stimulation Times.

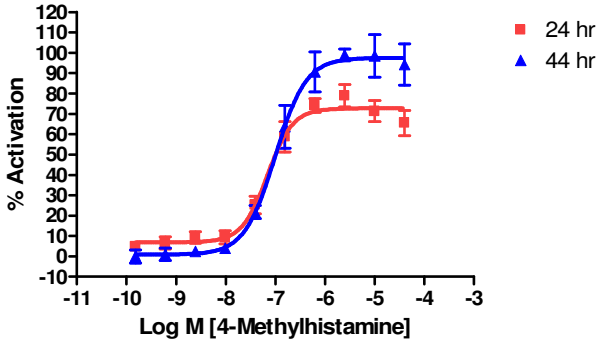
Figure4 — Tango™ H4-*bla* U2OS cells dose response to 4-Methylhistaminewith 5 hr or 16 hr stimulation times.



Tango™H4-*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. 4-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. Emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the Response Ratio plotted against the indicated concentrations of 4-Methylhistamine. Although the assay window is much larger at 16 hrs a suitable response is obtained at 5 hrs.

Assay Performance with Variable Serum Starve Times

Figure 5– Tango™ H4-*bla* U2OS cells dose response to 4-Methylhistamine with 24 or 48 hour serum starve times



Tango™H4-*bla* U2OS cells (10,000 cells/well) were plated either 24 or 48 hours prior to assay in a 384-well assay plate. 4-Methylhistamine (Tocris 2342) was then added to the plate over the indicated concentration range for 5 hrs in 0.1% DMSO. The cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. 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 4-Methylhistamine.

References

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

2) 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).