Tango™ CHRM2-*bla* U2OS DA Assay Kit

Tango™ CHRM2-*bla* U2OS cells

Catalog Numbers – K1565 and K1564

Cell Line Descriptions

Tango™ CHRM2-*bla* U2OS DA (Division Arrested) cells and Tango™ CHRM2-*bla* U2OS cells contain the human Cholinergic muscarinic 2 receptor (CHRM2) 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 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 the Tango™ CHRM2-*bla* U2OS cells and the Tango™ CHRM2-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of Carbachol (Figure 1). In addition, Tango™ CHRM2-*bla* U2OS cells have been tested for assay performance under variable conditions.

Validation Summary

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

1. Carbachol dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	6.5 µM	14 µM
Z'-factor	0.76	0.69
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		= 1 mM

2. Antagonist dose response

Scopolamine IC_{50} = 65.2 nM

Assay Testing Summary

3. Assay performance with variable stimulation time.

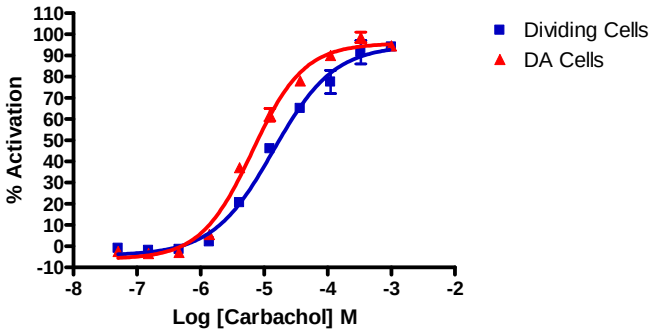
Carbachol 5 Hr. EC_{50} = 6.6uM
Carbachol 16Hr. EC_{50} = 50uM

4. Assay performance in 2nd messenger assay.

Carbachol EC_{50} = 14.75uM

Primary Agonist Dose Response

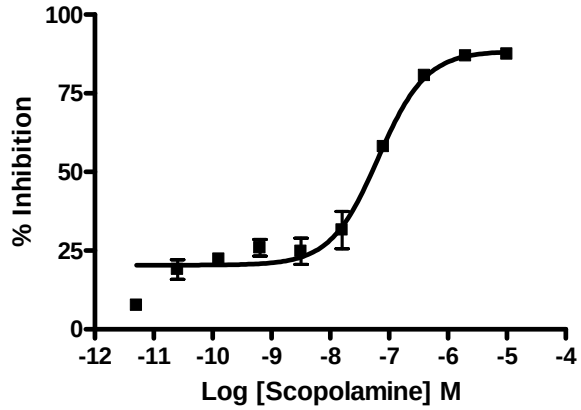
Figure 1 — Tango™ CHRM2-*bla* U2OS cells and Tango™ CHRM2-*bla* U2OS DA cells dose response to Carbachol under optimized conditions



Tango™ CHRM2-*bla* U2OS cells and Tango™ CHRM2-*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 Carbachol (Sigma C4382) 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 Carbachol.

Antagonist Dose Response

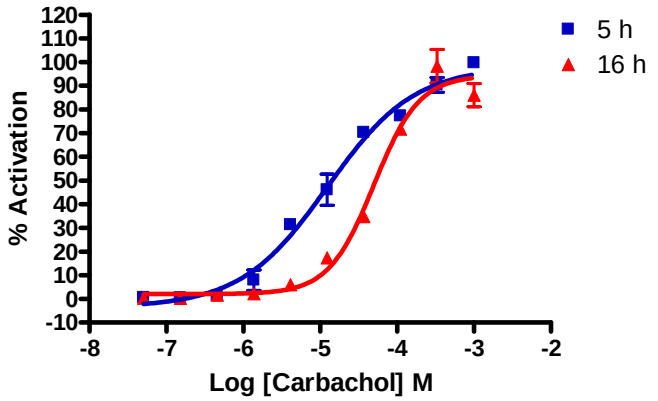
Figure 2 — Tango™ CHRM2-*bla* U2OS cells dose response to Scolopamine



Tango™ CHRM2-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to Scolopamine (Sigma S1875) for 30 min. and then stimulated with an EC80 concentration of Carbachol (Sigma C4382) 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 Scolopamine.

Assay Performance with Variable Stimulation Time

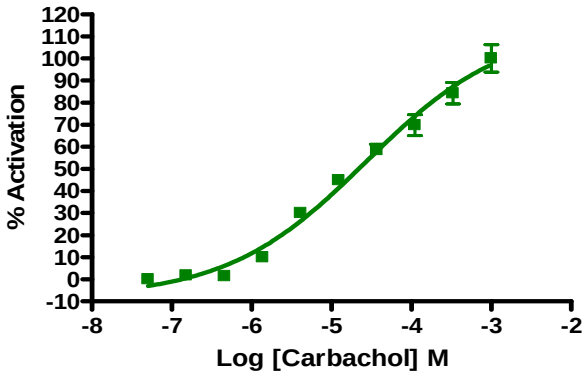
Figure 3 – Tango™ CHRM2-*bla* U2OS cells dose response to Carbachol with 5 or 16 hour stimulation times



CHRM2-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well plate and incubated for 16-24 hours. Carbachol (Sigma C4382) 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 the % Activation plotted against the indicated concentrations of Carbachol.

Agonist 2nd Messenger Dose Response

Figure 4 — Tango™ CHRM2-*bla* U2OS 2nd messenger dose response to Carbachol under optimized conditions.



Tango™ CHRM2-*bla* U2OS cells were loaded with Fluo4-AM and tested for a response to carbachol. The % Activation was plotted against the indicated concentrations of Carbachol.