Tango™ ADRA2A-*bla* U2OS DA cells

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Catalog Numbers – K1607 and K1604

Cell Line Descriptions

Tango™ ADRA2A-*bla* U2OS DA (Division Arrested) cells and Tango™ ADRA2A-*bla* U2OS cells contain the human Adrenergic Alpha-2A Receptor (ADRA2A) 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.

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™ ADRA2A-*bla* U2OS cells and the Tango™ ADRA2A-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of UK14,304 (Figure 1). In addition, Tango™ ADRA2A-*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. UK14,304 dose response under optimized conditions

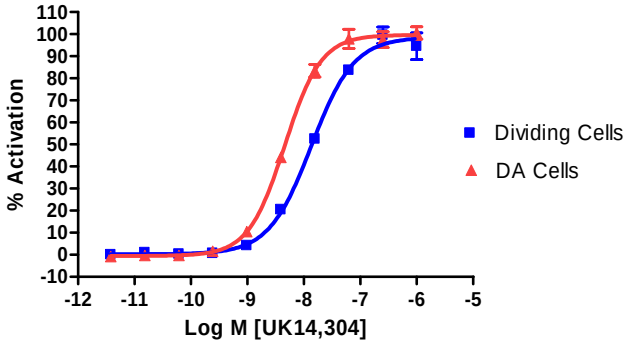
	DA cells	Dividing Cells
EC_{50}	4.6 nM	13.6 nM
Z'-factor	0.78	0.51
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		= 1,000 nM

2. Antagonist dose response

Yohimbine:	
Dividing IC_{50}	= 8.34 nM
Cryopreserved IC_{50}	= 7.77 nM
DA IC_{50}	= 6.79 nM

Primary Agonist Dose Response

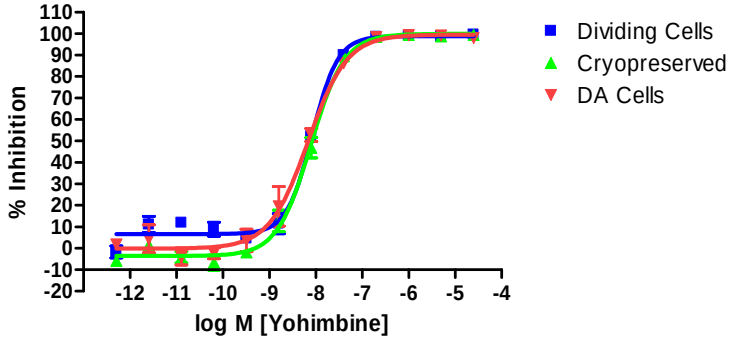
Figure 1 — Tango™ ADRA2A-*bla* U2OS cells and Tango™ ADRA2A-*bla* U2OS DA cells dose response to UK14,304 under optimized conditions



Tango™ ADRA2A-*bla* U2OS cells and Tango™ ADRA2A-*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 UK14,304 (Sigma U104) 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 UK14,304.

Antagonist Dose Response

Figure 3 — Tango™ ADRA2A-*bla* U2OS cells dose response to Yohimbine



Tango™ ADRA2A-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to Yohimbine (Sigma Y3125) for 30 min. and then stimulated with an EC80 concentration of UK14,304 (Sigma U104) 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 Yohimbine.