Optimization of the Tango™ ADORA1-*bla* U2OS Cell Line

Tango™ ADORA1-*bla* U2OS DA cells

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Catalog Numbers – K1601 and K1445

Cell Line Descriptions

Tango™ ADORA1-*bla* U2OS DA (Division Arrested) cells and Tango™ ADORA1-*bla* U2OS cells contain the human Adenosine A1 receptor (ADORA1) 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 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™ ADORA1-*bla* U2OS cells and the Tango™ ADORA1-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} ceoncentrations of a NECA (Figure 1). In addition, Tango™ ADORA1-*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. NECA dose response under optimized conditions

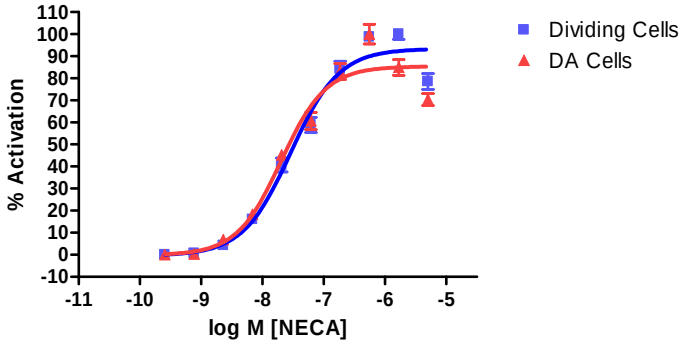
	DA cells	Dividing Cells
EC_{50}	20.24 nM	26.3 nM
Z'-factor	0.67	0.72
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		=5,000 nM

2. Antagonist dose response

DPCPX (Dividing) IC_{50}	= 6.3 nM
DPCPX (Cryopreserved) IC_{50}	= 8.1 nM
DPCPX (DA) IC_{50}	= 7.4 nM

Primary Agonist Dose Response

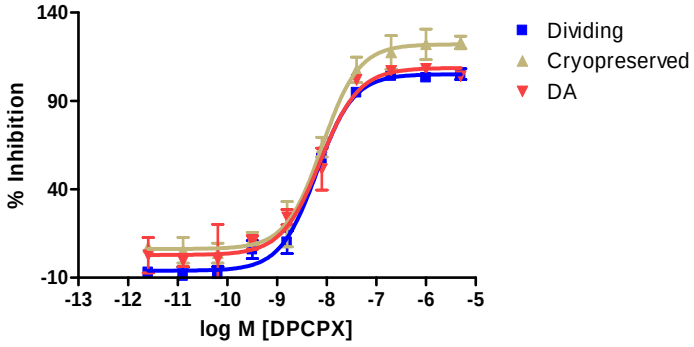
Figure 1 — Tango™ ADORA1-*bla* U2OS cells and Tango™ ADORA1-*bla* U2OS DA cells dose response to NECA under optimized conditions



Tango™ ADORA1-*bla* U2OS cells and Tango™ ADORA1-*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 NECA (Sigma E2387) 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 NECA.

Antagonist Dose Response

Figure 3 — Tango™ ADORA1-*bla* U2OS cells dose response to DPCPX



Tango™ ADORA1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to DPCPX (Tocris 439) for 30 min. and then stimulated with an EC80 concentration of NECA (Sigma E2387) 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 DPCPX.