**Tango™ D1-*bla* U2OS DA cells**

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Catalog Numbers – K1505 and K1485

## Cell Line Descriptions

Tango™ D1-*bla* U2OS DA (Division Arrested) cells and Tango™ D1-*bla* U2OS cells contain the human Dopamine Receptor 1 (D1) 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™ D1-*bla* U2OS cells and the Tango™ D1-*bla* U2OS DA cells have been functionally validated for Z' factor and $EC_{50}$ concentrations of Dihydrexidine (Figure 1). In addition, Tango™ D1-*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. Dihydrexidine dose response under optimized conditions

| | DA cells | Dividing Cells |
|---|---|---|
| $EC_{50}$ | 400 nM | 249 nM |
| Z'-factor | 0.77 | 0.76 |
| Recommended cell no. /well | | = 15,000 |
| Recommended Stim. Time | | = 5 hrs |
| Max. [Stimulation] | | = 25,000 nM |

### 2. Antagonist dose response

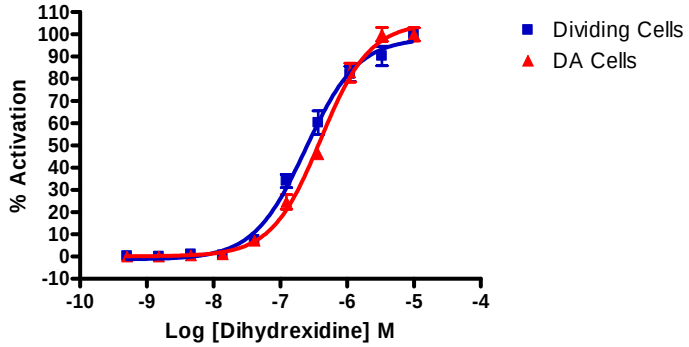
R(+)-SCH-23390 $IC_{50}$ = 2.1 nM

### 3. Agonist 2nd messenger dose response.

Dihydrexidine $EC_{50}$ = 15 nM

## Primary Agonist Dose Response

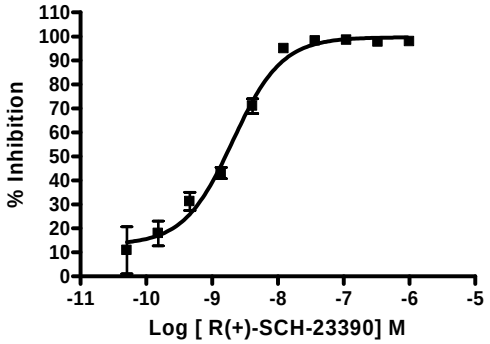
**Figure 1 — Tango™ D1-*bla* U2OS cells and Tango™ D1-*bla* U2OS DA cells dose response to Dihydrexidine under optimized conditions**




Tango™ D1-*bla* U2OS cells and Tango™ D1-*bla* U2OS DA cells (15,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of Dihydrexidine (Tocris 884) 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.

## Antagonist Dose Response

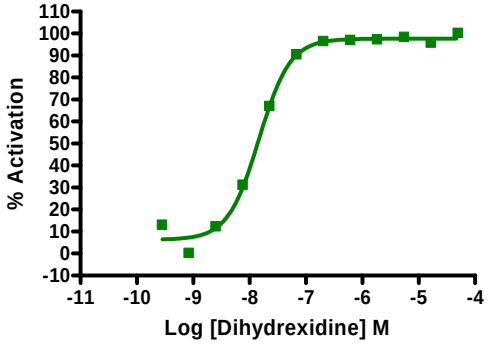
**Figure 2 — Tango™ D1-*bla* U2OS cells dose response to R (+)-SCH-23390**




Tango™ D1-*bla* U2OS cells (15,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to ( ) for 30 min. and then stimulated with an EC80 concentration of Dihydrexidine (Tocris 884) 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 R(+)-SCH-23390

## 2nd Messenger Dose Response

**Figure 3 — Tango D1-*bla* U2OS cells 2nd messenger dose response to Dihydrexidine under optimized conditions.**




Tango™ D1-*bla* U2OS cells were tested for a response to dihydrexidine with a TR-FRET cAMP kit.