Tango™ OPRK1-*bla* U2OS cells

Catalog Numbers – K1576

Cell Line Descriptions

Tango™ OPRK1-*bla* U2OS cells contain the human Opioid receptor kappa 1 (OPRK1) 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™ OPRK1-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of U-50,488 (Figure 1). In addition, Tango™ OPRK1-*bla* U2OS cells have been tested for assay performance under variable conditions.

Target Description

Opioids have been known analgesics and anti-diuretics for over 5000 years. However, it wasn't until 1954 that Beckett and Casy discovered the opioid receptors in the body (1). Experiments on opioid receptors in the 60's and 70's led scientists to believe that there is more than one type of opioid receptor in the body (2, 3). The receptors were classified as μ (mu), κ (kappa) and δ (delta) after the drugs (morphine, ketocyclazocine and deferense, respectively) that were used to differentiate these receptors (4). The cloning of these opioid receptor sub-types also gave rise to the discovery of the Opioid Receptor like 1 (OPRL1) receptor (5).

Opioid Receptor Kappa 1 (OPRK1) is known to regulate pain and pain perception. The OPRK1 receptors are involved in the production of urine by the kidneys leading to the increased occurrence of urination. OPRK1 is also found to regulate feeding, inhibit neurotransmitter release, and regulate temperature and modulate cardio respiratory function. (6-7). The OPRK1 agonists also cause dysphoria which can lead to addiction as users seek the high.

OPRK1 receptors are found mostly in the brain with the highest levels in the cerebral cortex, hypothalamus and nucleus accumbens (8, 9). The receptors are also found in the gastrointestinal tract, immune cells and various peripheral tissues.

Validation Summary

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

1. U-50,488 dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	2.88 nM	5.2 nM
Z'-factor	0.77	0.84
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		= 150 nM

2. Antagonist dose response

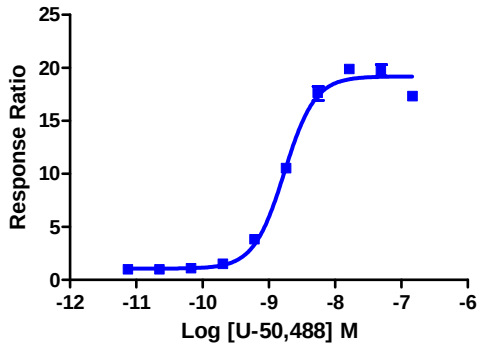
Nor-BP dihydrochloride:	
Dividing IC_{50}	= 0.41 nM
Cryopreserved IC_{50}	= 0.55 nM
Division Arrested IC_{50}	= 0.21 nM

3. Alternate agonist dose response

U-69593 EC_{50}	= 3.6 nM

Primary Agonist Dose Response

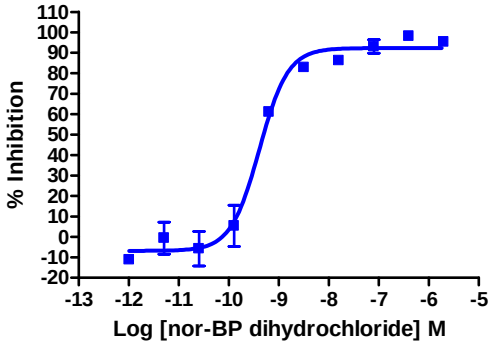
Figure 1 — Tango™ OPRK1-*bla* U2OS cells dose response to U-50,488 under optimized conditions



Tango™ OPRK1-*bla* U2OS cells and Tango™ OPRK1-*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 U-50,488 (Sigma D8040) 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 U-50,488.

Antagonist Dose Response

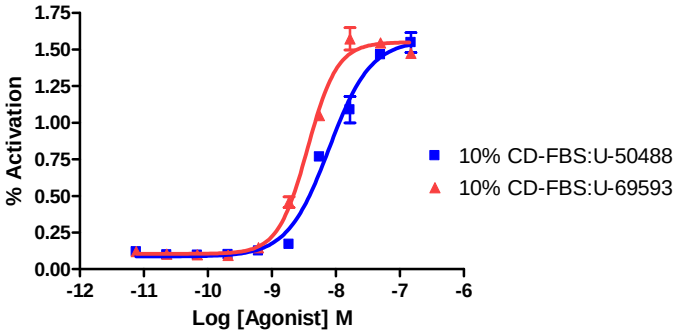
Figure 2 — Tango™ OPRK1-*bla* U2OS cells dose response to nor-BP dihydrochloride



Tango™ OPRK1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to nor-BP dihydrochloride (Tocris 347) for 30 min. and then stimulated with an EC80 concentration of U-50,488 (Sigma D8040) 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 nor-BP dihydrochloride.

Alternate Agonist Dose Response and Selectivity

Figure 3 — Tango™ OPRK1-*bla* U2OS cells dose response to U-50488, and U-69593.



Tango™ OPRK1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with U-50488 (Sigma D8040), and U-69593 (Sigma, U-103) 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.

References

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