Tango™ CNR2-*bla* U2OS DA Assay Kit

Tango™ CNR2-*bla* U2OS cells

Catalog Numbers – K1567 and K1512

Cell Line Descriptions

Tango™ CNR2-*bla* U2OS DA (Division Arrested) cells and Tango™ CNR2-*bla* U2OS cells contain the human Cannabinoid Receptor 2 (CNR2) 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™ CNR2-*bla* U2OS cells and the Tango™ CNR2-*bla* U2OS DA cells have been functionally validated for Z' factor and EC_{50} concentrations of CP-55940 (Figure 1). In addition, Tango™ CNR2-*bla* U2OS cells have been tested for assay performance under variable conditions.

Target Description

The cannabinoid receptors are a family of GPCRs named after their endogenous ligand Δ9-tetrahydrocannabinol (Δ^9 THC). Δ^9-THC is the psychoactive component of *Cannabis sativa* or marijuana. It wasn't until the 1980s that science found that the effects of marijuana are receptor based. The Cannabinoid 1 (CNR1) receptor was cloned and classified in 1990. The CNR1 receptor decreases cellular cAMP levels and regulation of L-, N- and Q-type calcium channels. CNR1 is responsible for the psychotropic affects of cannabis, regulation of appetite, nausea and vomiting (1-3). Cannabinoid 2 receptor (CNR2) was cloned and classified in 1993 and is 44% homologous to the CNR1 receptor. This receptor also decreases cellular cAMP levels but has no known affect on ion channels. The function of the CNR2 receptor is not certain however, it is thought to be involved in inflammation and immunomodulation (1-3). Currently, GPR55 is thought to be a third member of the Cannabinoid family as it exhibits binding to traditional cannabinoid ligands (1-3).

Validation Summary

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

1. CP-55940 dose response under optimized conditions

	DA cells	Dividing Cells
EC_{50}	38.08 nM	39.54 nM
Z'-factor	0.93	0.64
Recommended cell no. /well		= 10,000
Recommended Stim. Time		= 5 hrs
Max. [Stimulation]		= 2500 nM

2. Alternate agonist dose response

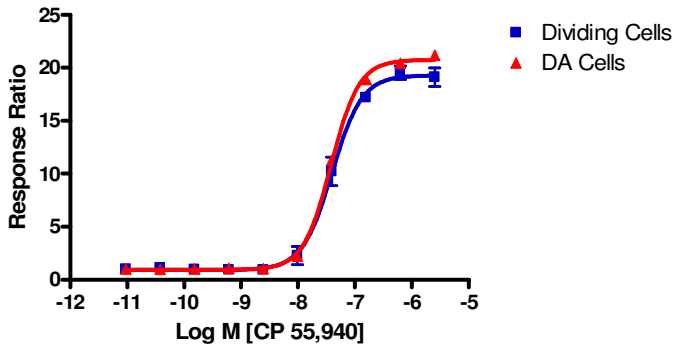
Win 55,212 EC_{50}	= 62.3 nM
JWH-015 EC_{50}	= 613.4 nM

3. Antagonist dose response

AM630 Live IC_{50}	= 3.74 µM
AM630 Cryo IC_{50}	= 1.85 µM
AM630 DA IC_{50}	= 3.82 µM

Primary Agonist Dose Response

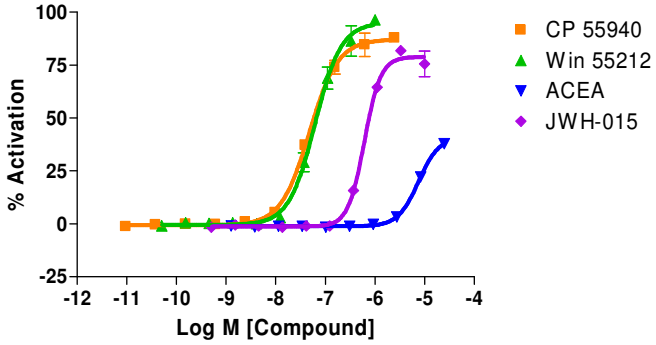
Figure 1 — Tango™ CNR2-*bla* U2OS cells and Tango™ CNR2-*bla* U2OS DA cells dose response to CP-55940 under optimized conditions



Tango™ CNR2-*bla* U2OS cells and Tango™ CNR2-*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 CP-55,940 (Sigma C1112) 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 Response Ratio plotted for each replicate against the concentrations of CP-55940.

Alternate Agonist Dose Response and Selectivity

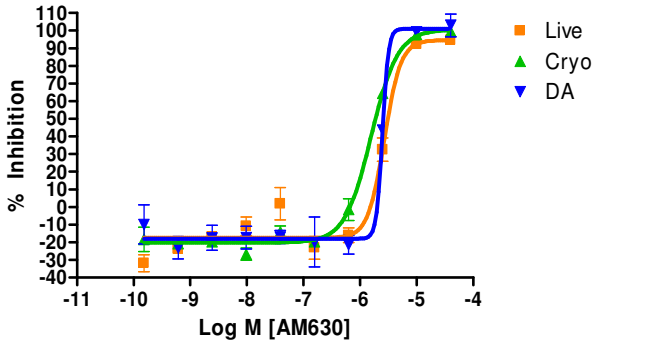
Figure 2 — Tango™ CNR2-*bla* U2OS cells dose response to CP-55940, Win 55,212, ACEA and JWH-015.



Tango™ CNR2-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with CP-55940 (Sigma C1112), Win 55,212 (Sigma W102), ACEA (Sigma A9719) and JWH-015 (Sigma, J4252) 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. The data shows the correct rank order potency and selectivity as ACEA is a CNR1 selective agonist.

Antagonist Dose Response

Figure 3 — Tango™ CNR2-*bla* U2OS cells dose response to AM630 using Live, Cryopreserved and Division Arrested Cells.



Tango™ CNR2-*bla* U2OS cells (both dividing and cryopreserved) and Tango™ CNR2-*bla* U2OS DA (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to AM630 (Tocris 1120) for 30 min. and then stimulated with an EC80 concentration of CP-55940 (Sigma C1112) 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 AM630.

References

1) Howlett, et al. **International Union of pharmacology. XXVII. Classification of cannabinoid receptors**. *Pharmacol. Rev.* **54** pp 161-202, (2002).

2) Howlett, et al. **Cannabinoid Receptors**. *The IUPHAR Compendium of Receptor Characterization and Classification, 2nd Edition.* Pp 129-138 (2000)

3) De Petrocellis, et al. **The endocannabinoid system: a general view and latest additions**. *Br. J. Pharmaco*. **141**, pp 765-774 (2004).