Tango™ CNR1-*bla* U2OS cells

Catalog Numbers – K1513

Cell Line Descriptions

Tango™ CNR1-*bla* U2OS cells contain the human Cannabinoid Receptor 1 (CNR1) 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.

The Tango™ CNR1-*bla* U2OS cells have been functionally validated for Z' factor and EC_{50} concentrations of CP-55,940 (Figure 1). In addition, Tango™ CNR1-*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).

CNR1 receptors are present in many tissues throughout the body. The highest concentration of cannabinoid receptors are in the neurons of the brain, the hippocampus, cerebral cortex, basal ganglia and the cerebellum (7). Low level expression occurs in the lung, testis and uterus, as well as vascular tissue (1-2).

Δ^9-THC and CP, 55940 are endogenous agonists of the CNR1 pathway. Several other Δ^9 THC and CP-55,940 analogs have been synthesized, including: HU 210 and WIN 55,212-2 (4-6). The CNR1 and CNR2 receptors have equal affinity for Cannabinoid agonists but several antagonists have been synthesized which are sub-type selective. Examples of these antagonists are AM251, LY-320135 and SR 141716A (4-6).

Validation Summary

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

1. **CP-55,940 dose response under optimized conditions**

	Cryo cells	Dividing Cells
EC_{50}	2.33 nM	3.4 nM
Z'-factor	0.75	0.69
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}	= 99 pM
ACEA EC_{50}	=728 nM

3. **Antagonist dose response**

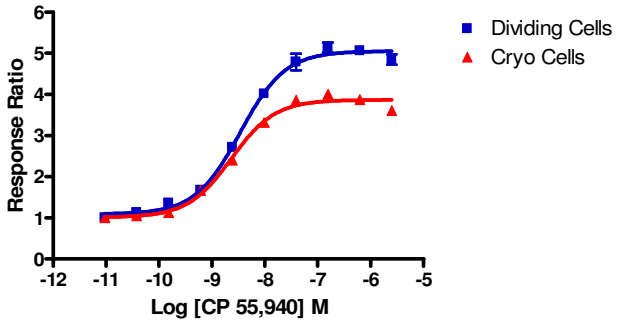
LY 320135 IC_{50}= 244 nM

Assay Testing Summary

4. **Assay performance with variable serum starve times.**

Primary Agonist Dose Response

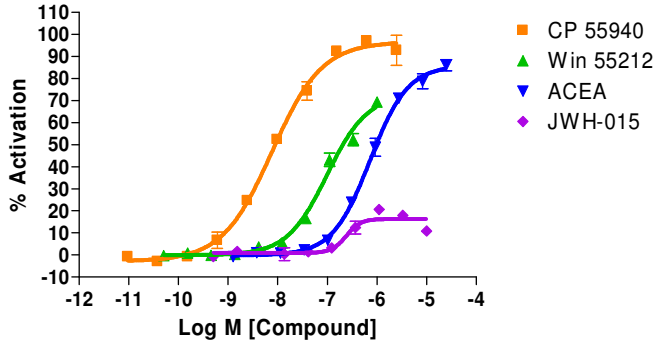
Figure 1 — Tango™ CNR1-*bla* U2OS dividing and cryopreserved cells dose response to CP-55,940 under optimized conditions



Tango™ CNR1-*bla* U2OS cells both live and cryopreserved (10,000 cells/well) were plated in a 384-well format and incubated for 44-48 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-55,940.

Alternate Agonist Dose Response and Selectivity

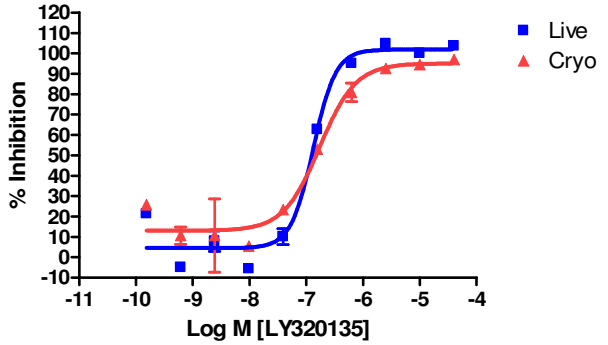
Figure 2 — Tango™ CNR1-*bla* U2OS cells dose response to CP55,940, Win 55,212, ACEA and JWH-015.



Tango™ CNR1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 44-48 hours prior to stimulation with CP 55,940 (Sigma C1112), Win 55,212 (Sigma W102), ACEA (Sigma A9719) and ACEA (Sigma, A9719) 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

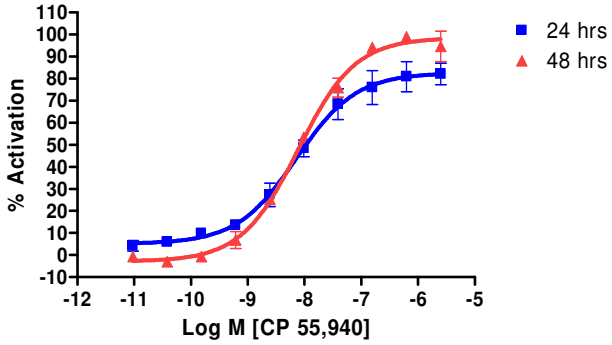
Figure 3 — Tango™ CNR1-*bla* U2OS cells dose response to LY 320135



Tango™ CNR1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 44-48 hours. Cells were exposed to LY 320135 (Tocris 2387) for 30 min. and then stimulated with an EC80 concentration 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 for the various substrate loading times were obtained using a standard fluorescence plate reader and the % Inhibition plotted against the indicated concentrations of LY 320135.

Assay Performance with Variable Serum Starve Times

Figure 4 – Tango™ CNR1-*bla* U2OS cells dose response to CP-55,940 with 24 or 48 hour serum starve times



Tango™ CNR1-*bla* U2OS cells (10,000 cells/well) were plated either 24 or 48 hrs prior to assay in a 384-well assay plate. CP-55,940 (Sigma C1112) was then added to the plate over the indicated concentration range for 5 hrs in 0.1% DMSO. The cells were then loaded for 2 hours with LiveBLAzer™-FRET B/G Substrate. 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 CP-55,940.

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).

4. Felder, and Glass. **Cannabinoid receptors and their endogenous agonists.** *Annu. Rev. Pharmacol. Toxicol.* **38**, pp 179-200 (1998).

5. Pertwee. **Pharmacology of cannabinoid receptor ligands.** *Curr. Med. Chem.* **6**, pp 635-664 (1999).

6. Pertwee, (ed.) **Cannabinoids. Handbook of Experimental Pharmacology.** Volume 168, Springer, Heidelberg. (2005).

7. Herkenham, et al. **Cannabinoid receptor localization in brain.** *Proc. Natl. Acad. Sci. U.S.A.* **87** pp 1932-1936 (1990).