
Optimization of the Tango™ MCHR2-*bla* U2OS Cell Line

Tango™ MCHR2-*bla* U2OS cells

Catalog Numbers – K1836

Cell Line Descriptions

Tango™ MCHR2-*bla* U2OS cells contain the human Melanin-concentrating Hormone 2 (GPR145) (MCHR2) 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.

The Tango™ MCHR2-*bla* U2OS cells have been functionally validated for Z' factor and EC₅₀ concentrations of Melanin Concentrating Hormone (6-17) (Figure 1). In addition, Tango™ MCHR2-*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. Melanin Concentrating Hormone (6-17) dose response under optimized conditions

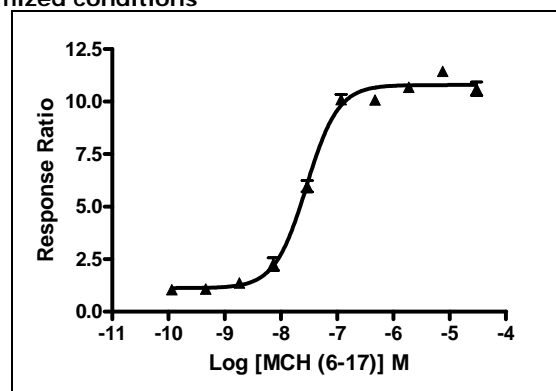
	<u>Cryo Cells</u>
EC ₅₀	28.3
Z'-factor	0.73
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 16 hrs
Max. [Stimulation]	= 30000 nM

Assay Testing Summary

2. Assay performance in 2nd messenger assay.

Primary Agonist Dose Response

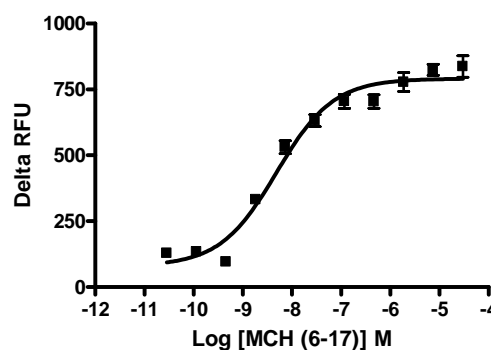
Figure 1 — Tango™ MCHR2-*bla* U2OS cells dose response to Melanin Concentrating Hormone (6-17) under optimized conditions



Tango™ MCHR2-*bla* U2OS 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 Melanin Concentrating Hormone (6-17) (Sigma M2567) in the presence of 0.1% DMSO for 16 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 Ratios plotted for each replicate against the concentrations of Melanin Concentrating Hormone (6-17).

2nd Messenger Dose Response

Figure 2 — Tango™ MCHR2-*bla* U2OS 2nd messenger dose response to Melanin Concentrating Hormone (6-17) under optimized conditions.



Tango™ MCHR2-*bla* U2OS cells were loaded with Fluo4-AM and tested for a response to Melanin Concentrating Hormone (6-17).