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**Optimization of the Tango™ TACR1-*bla* U2OS Cell Line**

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**Tango™ TACR1-*bla* U2OS cells**

Catalog Numbers – K1801

**Cell Line Descriptions**

Tango™ TACR1-*bla* U2OS cells contain the human Tachykinin 1 (TACR1) 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™ TACR1-*bla* U2OS cells have been functionally validated for Z' factor and EC<sub>50</sub> concentrations of a (SAR9, MET(02)11)-Substance P (Figure 1). In addition, Tango™ TACR1-*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. (SAR9,MET(02)11)-Substance P dose response under optimized conditions

	<u>Dividing Cells</u>
EC <sub>50</sub>	1.711 nM
Z'-factor	0.75
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 312.5 nM

### 2. Alternate agonist dose response

Substance P EC<sub>50</sub> = 2.3 nM

### 3. Antagonist dose response

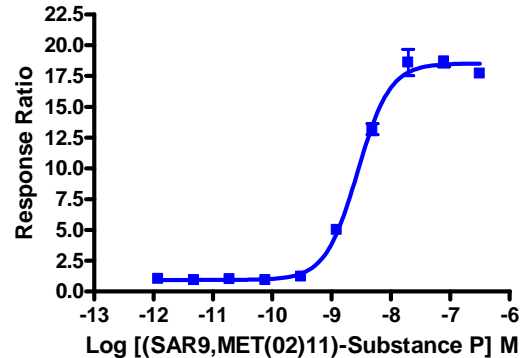
SDZ NKT 343IC<sub>50</sub> = 964 pM

## Assay Testing Summary

4. Assay Performance with variable cell number.
5. Assay Performance with variable stimulation time.

## Primary Agonist Dose Response

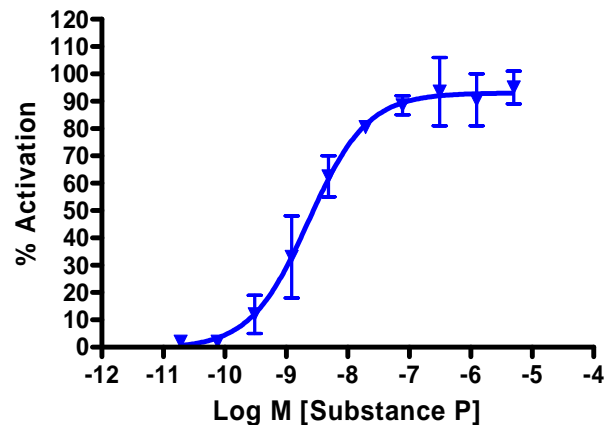
**Figure 1 — Tango™ TACR1-*bla* U2OS cells dose response to (SAR9,MET(02)11)-Substance P under optimized conditions**



Tango™ TACR1-*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 (SAR9,MET(02)11)-Substance P (Sigma S3672) 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 (SAR9,MET(02)11)-Substance P.

## Alternate Agonist Dose Response

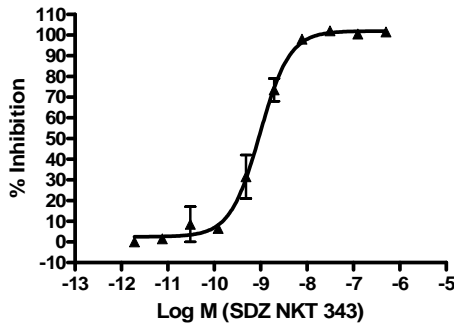
**Figure 2 — Tango™ TACR1-*bla* U2OS cells dose response to Substance P.**



Tango™ TACR1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with Substance P (Sigma #S6883) 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.

## Antagonist Dose Response

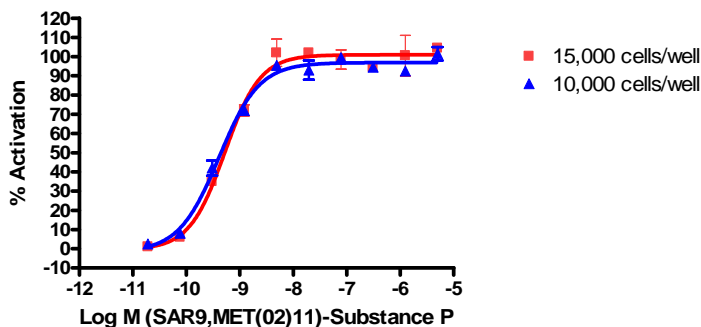
Figure 3 — Tango™ TACR1-*bla* U2OS cells dose response to SDZ NKT 343



Tango™ TACR1-*bla* U2OS cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to SDZ NKT 343 (Tocris #2394) for 60 min. and then stimulated with an EC80 concentration of (SAR9,MET(02)11)-Substance P (Sigma S3672) 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 agonist.

## Assay Performance with Variable Cell Number

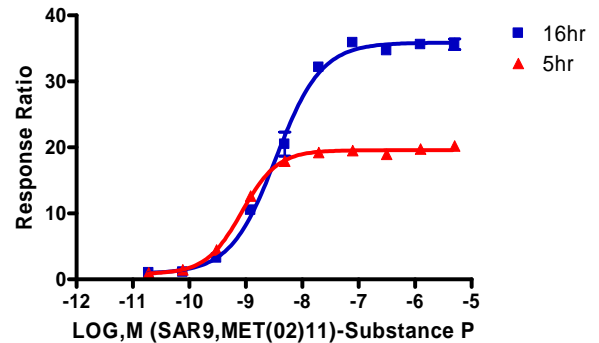
Figure 4 — Tango™ TACR1-*bla* U2OS cells dose response to (SAR9,MET(02)11)-Substance P with 10K or 15K cells/well



Tango™ TACR1-*bla* U2OS cells cells were plated in a 384-well format at 10,000 or 15,000 cells/well and incubated for 16-24 hours. On the day of the assay, cells were stimulated with (SAR9,MET(02)11)-Substance P (Sigma S3672) in the presence of 0.1% DMSO for 5hours. 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 (SAR9,MET(02)11)-Substance P.

## Assay Performance with Variable Stimulation Time

Figure 5 — Tango™ TACR1-*bla* U2OS cells dose response to (SAR9,MET(02)11)-Substance P with 5 or 16 hour stimulation times



TACR1-*bla* U2OS cells (10,000 cells/well) were plated the day before the assay in a 384-well assay plate. (SAR9,MET(02)11)-Substance P (Sigma S3672) was either added at the time of plating (for the 16 hour assay) or was added to for 5 hours after the overnight incubation (for the 5 hour assay). 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 Response Ratio plotted against the indicated concentrations of (SAR9,MET(02)11)-Substance P.