

Supplemental Material

Validation of a High-Throughput Calcium Mobilization Assay for the Human Trace Amine-Associated Receptor 1

By Ann M. Decker*, Kelly M. Mathews, Bruce E. Blough, and Brian P. Gilmour.

Center for Drug Discovery, RTI International, Research Triangle Park, North Carolina, 27709, USA

Correspondence: Dr. Ann M. Decker, Center for Drug Discovery, RTI International, Post Office Box 12194, Research Triangle Park, NC 27709, USA. Tel: 919-541-1246, E-mail: adecker@rti.org.

Parameter	Addition-2	Addition-3
Z'-factor	0.84 ± 0.09	0.79 ± 0.07
S/N	87 ± 15	253 ± 85
%CV	4 ± 3	6 ± 2

Table S1. Assay performance data for 384-well hTAAR1 calcium mobilization assay. Parameters were calculated as described in the Materials and Methods section. Each value is the mean ± S.D. from five independent experiments.

	PEA EC ₅₀ (nM) ± S.D., Addition-2	PEA EC ₅₀ (nM) ± S.D., Addition-3
Day 1	154 ± 36	123 ± 14
Day 2	145 ± 7	100 ± 20
Day 3	179 ± 15	140 ± 15
Day 4	104 ± 14	126 ± 24
Day 5	122 ± 11	118 ± 10

Table S2. Intra-day and inter-day data for 384-well hTAAR1 calcium mobilization assay. The PEA potency was monitored on six plates over five days. Each value is the mean ± S.D. from six separate plates. Results cluster well around the 151 nM EC₅₀ value determined from the 384-well miniaturization assays.

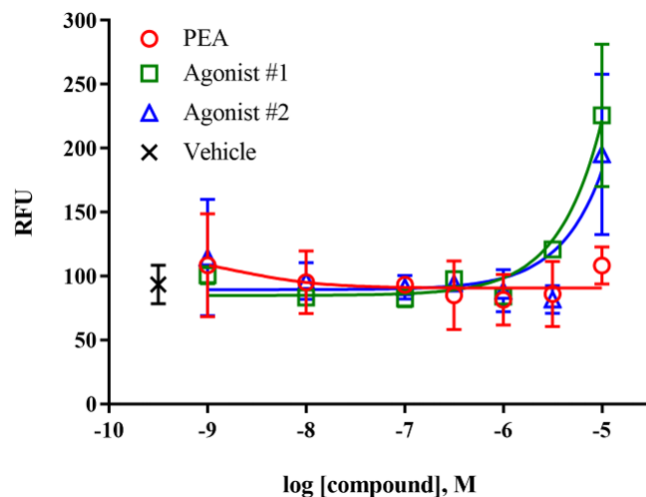


Figure S1. Response of two confirmed agonists in CHO-Gα_{q16} cells. Concentration response curves of two agonists and the control PEA in parental CHO-Gα_{q16} cells, as described in the Materials and Methods section. Data points are the mean ± S.D. of two independent experiments conducted in duplicate. PEA does not elicit a response while the two agonists elicit a minimal response (~200 RFUs).

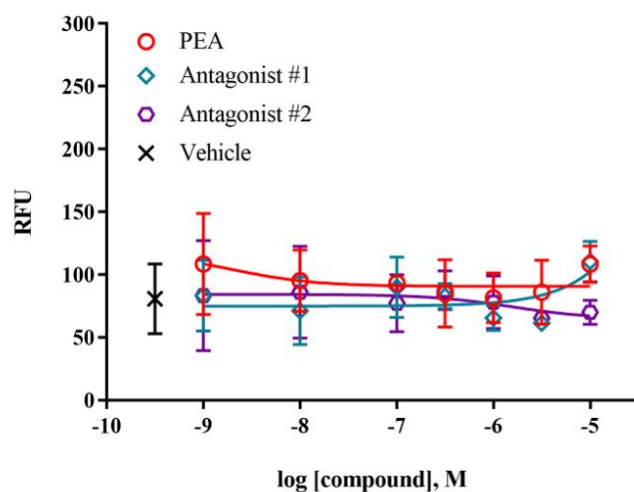


Figure S2. Response of two confirmed antagonist equivalents in CHO-Gα_{q16} cells. Concentration response curves of two agonists and the control PEA in parental CHO-Gα_{q16} cells, as described in the Materials and Methods section. Data points are the mean ± S.D. of two independent experiments conducted in duplicate. No response is elicited by either antagonist.

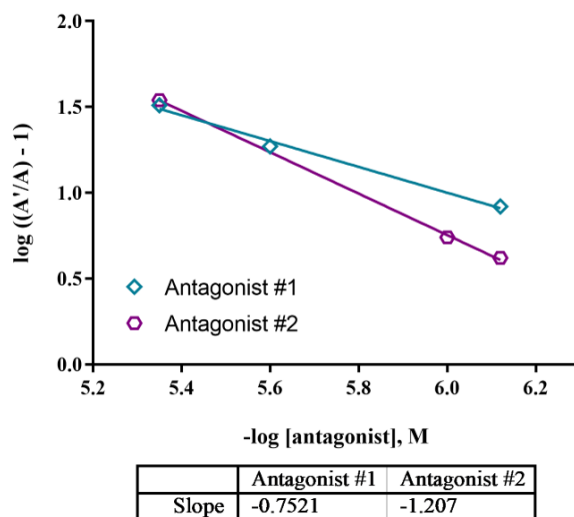


Figure S3. Schild plot for two confirmed antagonist equivalents in CHO-G α_{q16} -hTAAR1 cells. Curve shift assays were performed as described in the Materials and Methods section. Representative data are shown and each data point represents a separate curve shift assay performed in duplicate.

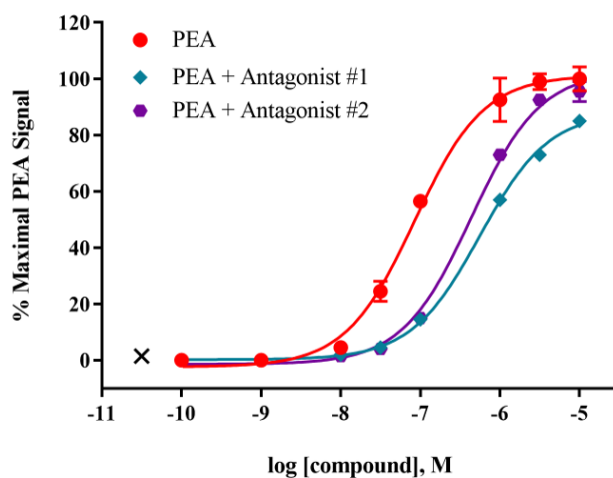


Figure S4. Curve shift assay to determine K_e values for two confirmed antagonist equivalents in CHO-G α_{q16} -hTAAR1 cells, as described in the Materials and Methods section. Representative data are shown and each data point is the mean \pm S.D. of duplicate determinations.