GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F Cells

Catalog Numbers – K1437

Cell Line Descriptions

GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F cells contain the human Calcitonin Gene Related Peptide Receptor (CALCRL:RAMP1), (Accession # NM_005795.3 NM_005855.2) stably integrated into the CellSensor® CRE-bla Freestyle™ 293F cell line. CellSensor® CRE-bla Freestyle™ 293F cells (Cat. no. K1636) contain a beta-lactamase (*bla*) reporter gene under control of the cAMP response element (CRE).

GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F cells are functionally validated for Z'-factor and EC_{50} concentrations of Alpha-CGRP (Figure 1). In addition, GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F 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. Alpha-CGRP dose response under optimized conditions

EC_{50}	= 135 pM
Z'-factor	= 0.80
Recommended cell no. /well	= 10,000
Recommended Stim. Time	= 5 hrs
Max. [Stimulation]	= 100 nM

2. Alternate agonist dose response

Beta-CGRP	= 118 pM

3. Antagonist dose response

Alpha-CGRP(8-37)	= 112 nM

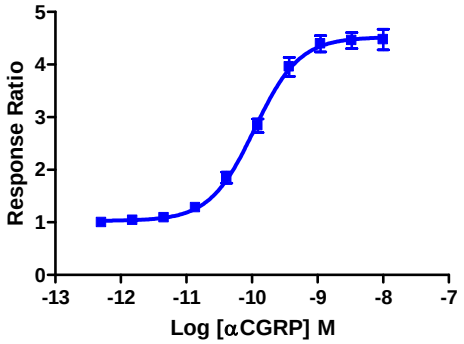
Assay Testing Summary

4. Assay performance in 2nd messenger assay.

Alpha-CGRP	= 64 pM

Primary Agonist Dose Response

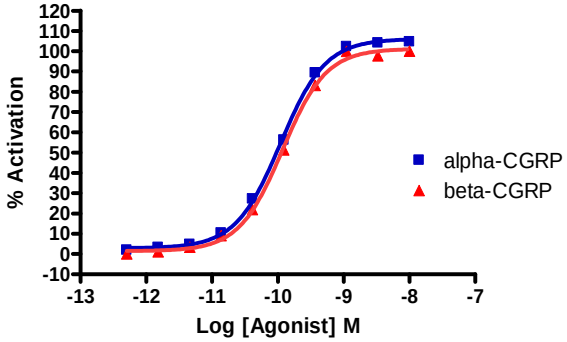
Figure 1 — GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F cells dose response to Alpha-CGRP under optimized conditions



GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F 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 Alpha-CGRP (Sigma C0167) 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 % Activation plotted for each replicate against the concentrations of Alpha-CGRP.

Alternate Agonist Dose Response

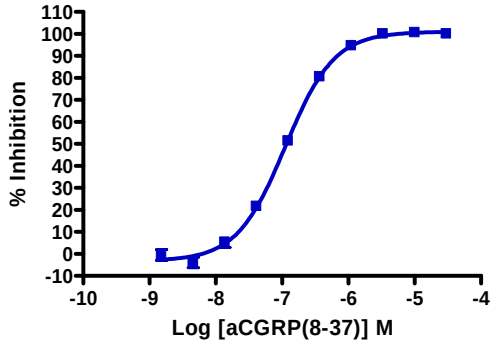
Figure 2 — GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F dose response to alpha-CGRP and beta-CGRP



GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours prior to stimulation with Alpha-CGRP (Sigma C0167), Beta-CGRP (Sigma C1044) 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.

Antagonist Dose Response

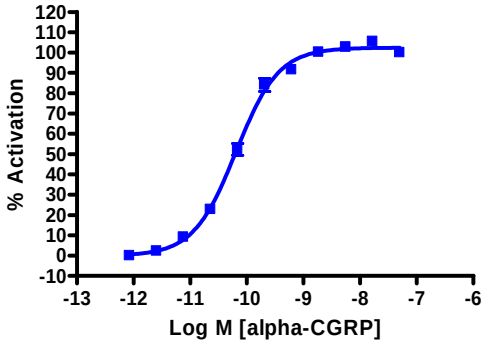
Figure 3 — GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F dose response to alpha-CGRP(8-37)



GeneBLAzer® CALCRL:RAMP1 CRE-*bla* FreeStyle™ 293F cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were exposed to alpha-CGRP(8-37) (Sigma C2806) for 30 min. and then stimulated with an EC80 concentration of Alpha-CGRP (Sigma C0167) 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 alpha-CGRP(8-37).

2nd Messenger Dose Response

Figure 4 — GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F 2nd messenger dose response to Alpha-CGRP under optimized conditions.



GeneBLAzer® CALCRL:RAMP1-CRE-*bla* FreeStyle™ 293F cells were tested for a response to Alpha-CGRP with a TR-FRET cAMP kit.