

GeneBLAzer® CALCRL:RAMP2-CRE-*bla* FreeStyle™ 293F Cells

Catalog Numbers – K1438

Cell Line Descriptions

GeneBLAzer® CALCRL:RAMP2-CRE-*bla* FreeStyle™ 293F cells contain the human Adrenomedullin Receptor 1 (CALCRL:RAMP2), (Accession #'s NM_005795.3, BC02795.1) 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:RAMP2-CRE-*bla* FreeStyle™ 293F cells are functionally validated for Z'-factor and EC₅₀ concentrations of Adrenomedullin(1-52) (Figure 1). In addition, GeneBLAzer® CALCRL:RAMP2-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. Adrenomedullin(1-52) dose response under optimized conditions

Dividing Cells

EC₅₀ = 920 pM
Z'-factor = 0.62

Recommended cell no. /well = 10,000
Recommended Stim. Time = 5 hrs
Max. [Stimulation] = 300 nM

2. Assay performance in 2nd messenger assay.

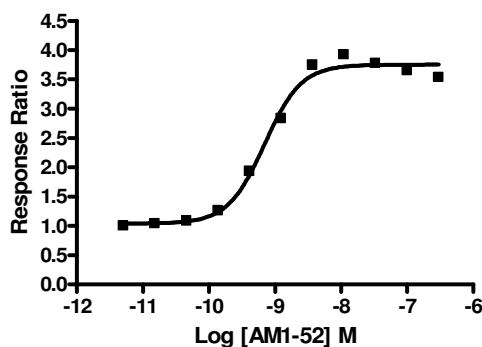
AM(1-52) = 2.6 nM

3. Antagonist dose response

AM(22-52) = 72 nM

Primary Agonist Dose Response

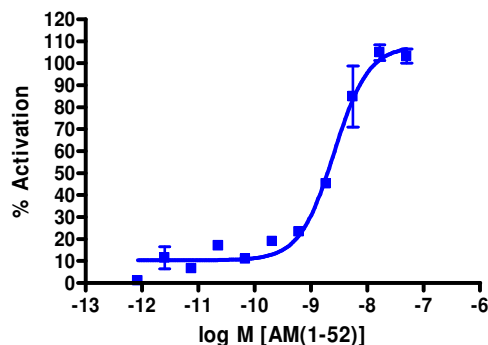
Figure 1 — GeneBLAzer® CALCRL:RAMP2-CRE-*bla* FreeStyle™ 293F cells dose response to Adrenomedullin(1-52) under optimized conditions



GeneBLAzer® CALCRL:RAMP2-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 Adrenomedullin(1-52) (Sigma A2327) 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 the response ratio was plotted for each replicate against the concentrations of Adrenomedullin(1-52).

2nd Messenger Dose Response

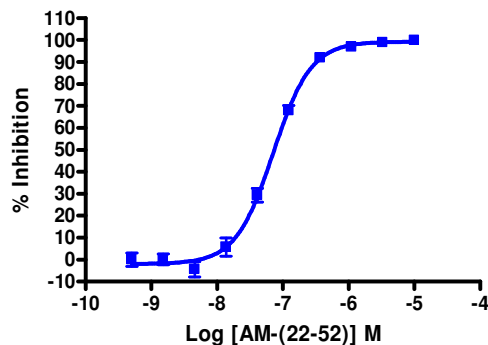
Figure 2 — GeneBLAzer® CALCRL:RAMP2-CRE-*bla* FreeStyle™ 293F 2nd messenger dose response to Adrenomedullin(1-52) under optimized conditions.



GeneBLAzer® CALCRL:RAMP2-CRE-*bla* FreeStyle™ 293F cells were tested for a response to Adrenomedullin(1-52) with a TR-FRET cAMP kit.

Antagonist Dose Response

Figure 3 — GeneBLAzer® CALCRL:RAMP2-CRE-*bla* FreeStyle™ 293F dose response to AM(22-52)



GeneBLAzer® CALCRL:RAMP2-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 AM(22-52) (Sigma A3832) for 30 min. and then stimulated with an EC80 concentration of Adrenomedullin(1-52) (Sigma A2327) 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 AM(22-52).