

**GeneBLAzer® CALCRL:RAMP3-CRE-*bla* FreeStyle™ 293F Cells**

Catalog Numbers – K1439

**Cell Line Descriptions**

GeneBLAzer® CALCRL:RAMP3-CRE-*bla* FreeStyle™ 293F cells contain the human Adrenomedullin Receptor 2 (CALCRL:RAMP3) (Accession # NM\_005795.3 NM\_005856.1) 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:RAMP3-CRE-*bla* FreeStyle™ 293F cells are functionally validated for Z'-factor and EC<sub>50</sub> concentrations of Adrenomedullin(1-52) (Figure 1). In addition, GeneBLAzer® CALCRL:RAMP3-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

EC<sub>50</sub> = 506 pM  
Z'-factor = 0.81

Recommended cell no. /well = 10,000  
Recommended Stim. Time = 5 hrs  
Max. [Stimulation] = 100 nM

### 2. Antagonist dose response

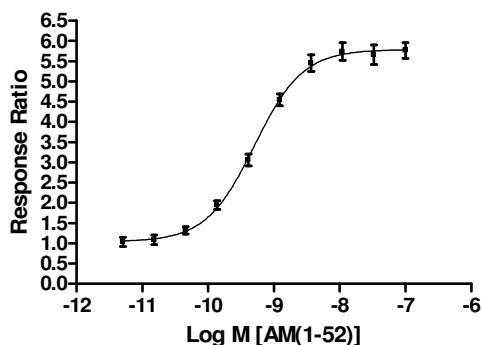
AM(22-52) = N/A

### 3. Assay performance in 2<sup>nd</sup> messenger assay.

AM(1-52) = 632 pM

## Primary Agonist Dose Response

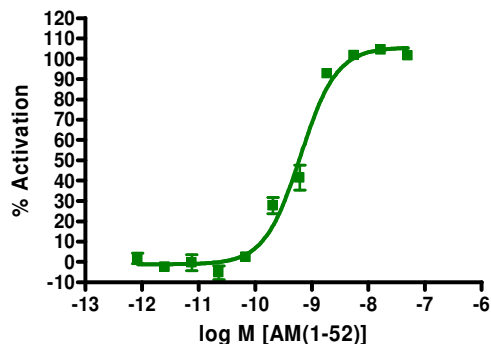
**Figure 1 — GeneBLAzer® CALCRL:RAMP3-CRE-bla FreeStyle™ 293F cells dose response to Adrenomedullin(1-52) under optimized conditions**



GeneBLAzer® CALCRL:RAMP3-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 % Activation plotted for each replicate against the concentrations of Adrenomedullin(1-52).

## 2<sup>nd</sup> Messenger Dose Response

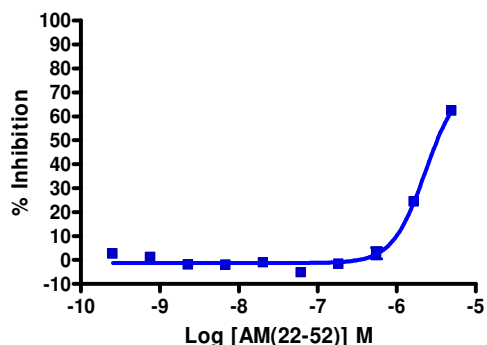
**Figure 2 — GeneBLAzer® CALCRL:RAMP3-CRE-bla FreeStyle™ 293F 2<sup>nd</sup> messenger dose response to Adrenomedullin(1-52) under optimized conditions.**



GeneBLAzer® CALCRL:RAMP3-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:RAMP3-CRE-bla FreeStyle™ 293F dose response to AM(22-52)**



GeneBLAzer® CALCRL:RAMP3-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).