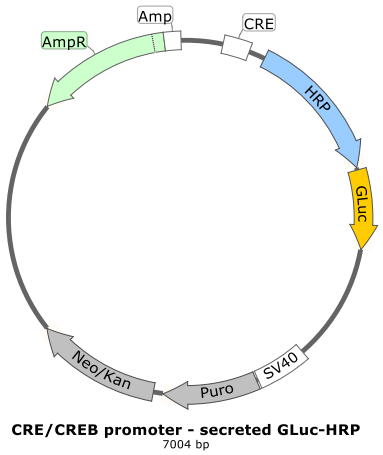
CRE/CREB – GLuc-HRP - cAMP Reporter

by InTouch BioSolutions

This cAMP reporter contains the control of multimerized cAMP response element (CRE) located upstream of the secreted Gaussia Luciferase (wt) - HRP (horseradish peroxidase) gene fusion. Expression of the GLuc-HRP fusion is induced by intracellular cAMP as a result of binding of the cAMP response element binding protein (CREB) to CRE. Secreted GLuc-HRP can be measured in the supernatant (see data below).

Plasmid details

- Response element: cAMP (cyclic adenosine monophosphate)
- Promoter containing CRE sites for CREB binding
- Reporter: Secreted Gaussia Luciferase (wt) - HRP (horseradish peroxidase) fusion protein
- Selection markers: SV40 promoter – Puromycin, Neomycin and Kanamycin resistance genes
- Plasmid size: ~7 kb (contains bacterial-derived sequences)
- Storage: -20°C. For long-term storage, the recommended storage is -80°C.



Application

- Expressed Gaussia Luciferase – HRP is secreted into the supernatant, thus cell lysis is not necessary to measure activity. Ideal for multiple sampling in time-dependent assays.
- Transcription regulation, virus-cell interactions, compound screening, post-translational modifications, GPCR signaling, cell signaling, promoter analysis.
- End-user preference on bioluminescence or colorimetric detection

Sample protocol

Approximately 1 million human melanoma cells were transfected with 10ug of CRE/CREB – GLuc-HRP plasmid by calcium phosphate method and seeded in a 96-well (5000 cells/well) flat bottom plate. The next day, cells were incubated with a GPCR agonist (alpha-MSH; 5nM) mixed with increasing amounts of inhibitor.

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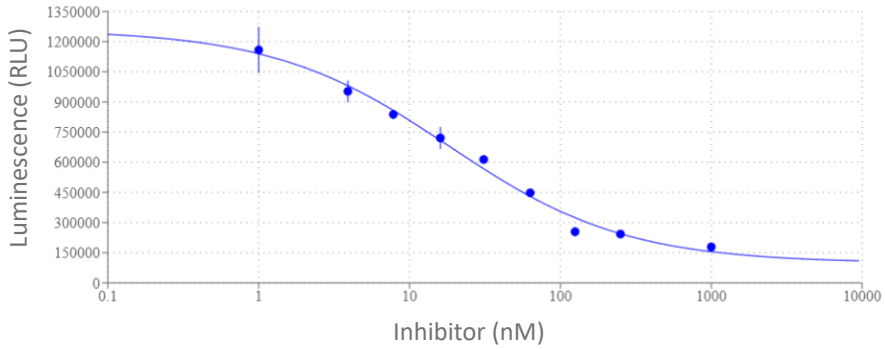
Data

A. Gaussia Luciferase activity



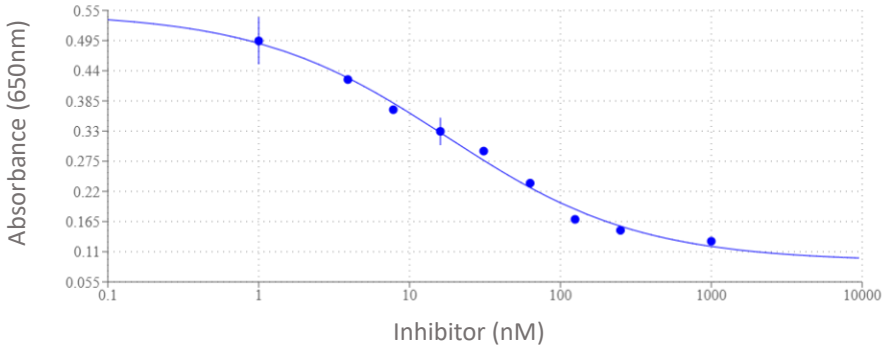
IC_{50} (nM) = 18.4

Max: 1,272,648
Min: 177,490

Error bar: SEM from duplicate wells

Figure A) After 24 hours, 10 µl from each well was added to wells containing native CTZ (Cat. #303, Nanolight Technology) and luminescence (RLU) measured using Molecular Devices Spectramax L plate reader.

B. HRP activity



IC_{50} (nM) = 17.7

Max: 0.537
Min: 0.127

Error bar: SEM from duplicate wells

Figure B) After 48 hours, 5 µl from each well was added to wells containing TMB substrate and the absorbance measured at 650 nm using Molecular Devices Thermomax plate reader. Inset is a representative image from the duplicate wells showing

Terms

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