

GloResponse™ Luciferase Reporter Cell Lines

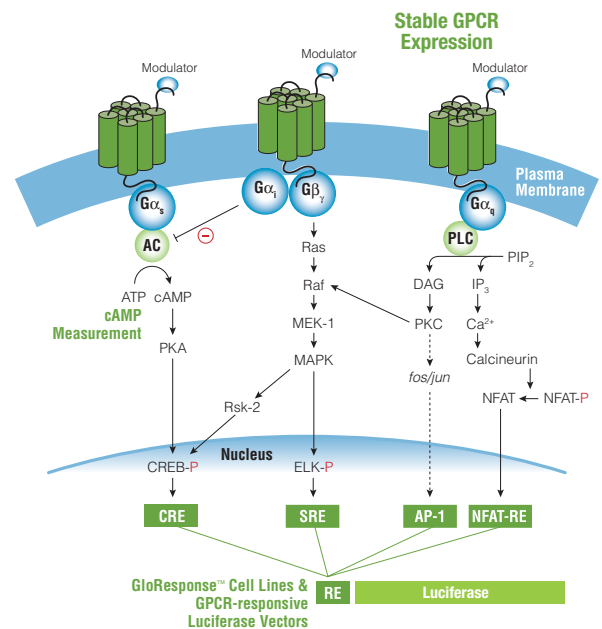
Description

Luciferase reporter assays have been widely used to investigate cellular signaling pathways and as high-throughput screening tools for drug discovery (1–3). The GloResponse™ NFAT-RE-*luc2P* HEK293 Cell Line and CRE-*luc2P* HEK293 Cell Lines contain state-of-the-art Luciferase technologies and are designed for rapid, convenient analysis of cell signaling through the nuclear factor of activated T-cells (NFAT) pathway or cAMP response pathways via activation of a luciferase reporter gene. Activity of non-native activators of these pathways (including GPCRs) can be studied after they have been introduced by transfection into the GloResponse™ Cell Lines.

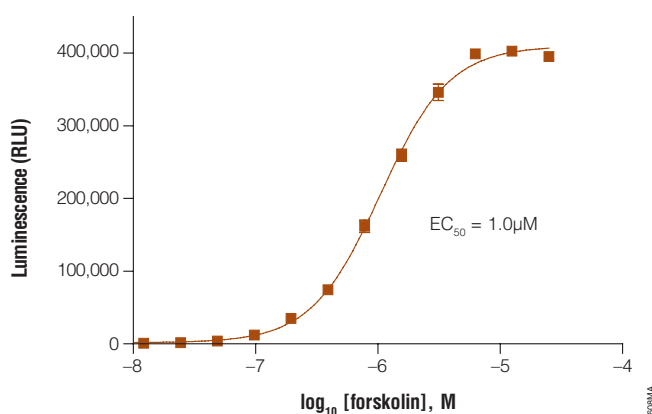
GPCR assays configured using the GloResponse™ Cell Lines are amenable to high-throughput screening. These luciferase assays typically have greater response dynamics (fold of induction) than other assay formats and good quality as indicated by high Z' values (4). GPCRs regulate a wide-range of biological functions and are one of the most important target classes for drug discovery (5). GPCR signaling pathways can be categorized into three classes based on the G protein α -subunit involved: G_s , $G_{i/o}$ and G_q . The GloResponse™ CRE-*luc2P* HEK293 Cell Line can be used to G_s - and $G_{i/o}$ -coupled GPCRs, which signal through cAMP and CRE. For G_q -coupled GPCRs, which signal through calcium ion and NFAT-RE, the GloResponse™ NFAT-RE-*luc2P* HEK293 Cell Line should be used.

Features

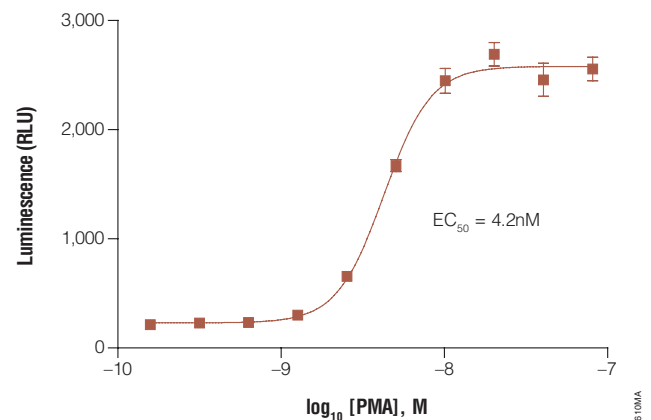
- Pre-built, optimized cell lines
- Large assay window from high levels of induction and low background expression
- Improved responsiveness to transcriptional dynamics



Schematic diagram showing GPCR signaling pathways. Upon stimulation, G_{α_s} -coupled receptors activate adenylyl cyclase (AC) resulting in an increase in cAMP; G_{α_i} -coupled receptors inhibit AC; the $\beta\gamma$ subunits activate the MAP kinase pathways; G_{α_q} -coupled receptors activate phospholipase C (PLC) to produce inositol triphosphate, which in turn increases intracellular calcium concentration. CRE, cAMP response element; SRE, serum response element; AP-1, activator protein 1, and NFAT-RE, nuclear factor of activated T-cell response element.

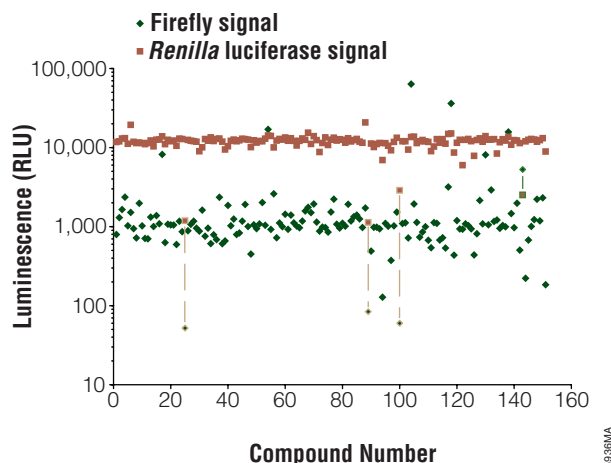


A total of 10,000 GloResponse™ CRE cells per well were dispensed into each well of a 96-well plate, and twofold serial dilutions of forskolin were added to induce reporter gene expression. After 4 hours of induction in a tissue culture incubator, luciferase activity was measured using the Dual-Glo™ Luciferase Assay System Reagent on the GloMax™ 96 Luminometer (Cat.# E6501; n = 4)



A total of 10,000 GloResponse™ NFAT-RE cells per well were dispensed into each well of a 384-well plate, and three fold serial dilutions of PMA were added to induce reporter gene expression. After 16 hours of induction in a tissue culture incubator, luciferase activity was quantified using the Dual-Glo™ Luciferase Assay System Reagent on the Berthold LB 96 V Luminometer. n = 8 for each data point.





Renilla luciferase as an internal control. A representative sample of the LOPAC screening in the NFAT/M3R cell line shows instances where the *Renilla* RLU values for certain samples are outside the range of the average RLU. In the wells that are highlighted yellow, both firefly and *Renilla* RLU are low. Highlighted in green is a well corresponding to a strong agonist in the NFAT signaling pathway where the low *Renilla* values may explain the lower-than-expected firefly values. In these cases, the *Renilla* value is an indicator that the firefly results are suspect and should be evaluated further.

The GloResponse™ Cell Lines were generated by clonal selection of HEK293 cells stably transfected with pGL4-based vectors carrying specific response elements for the pathway of interest. These cell lines incorporate the enhanced performance of the pGL4 vectors using destabilized *luc2P* luciferase reporter for improved responsiveness to transcriptional dynamics. The *luc2P* gene is codon-optimized for enhanced expression in mammalian cells, and the pGL4 plasmid backbone has been engineered to reduce background reporter expression. The result is a cell line with very high induction levels when the pathway of interest is activated.

References

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2. Zhuang, F. and Liu, Y.H. (2006) Usefulness of the luciferase reporter system to test the efficacy of siRNA. *Methods Mol. Biol.* **342**, 131–7.
3. Hill, S.J., Baker, J.G. and Rees, S. (2001) Reporter-gene systems for the study of Gprotein-coupled receptors. *Curr. Opin. Pharmacol.* **1**, 526–32.
4. Zhang, J-H., Chung, T.D.Y. and Oldenburg, K.R. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* **4**, 67–73.
5. Klubunde, T. and Hessler, G. (2002) Drug design strategies for targeting G-protein coupled receptors. *Chembiochem.* **3**, 928–44.

Ordering Information

Product	Size	Cat. #
GloResponse™ CRE- <i>luc2P</i> HEK293 Cell Line	each	E8500
GloResponse™ NFAT-RE- <i>luc2P</i> HEK293 Cell Line	each	E8510

Use of Genetically Modified Microorganisms (GMM)

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HEK293 cells were obtained under license from AdVec Inc.

Commercial use of this cell line requires a license from AdVec Inc.

The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

The NFAT response element and its use are licensed under one or more of the following patents: U.S. Pat. Nos. 5,837,840, 6,197,925, 5,989,810, 6,096,515, 6,388,052, 6,150,099, 6,171,781, 6,352,830, 6,312,899 and 6,875,571.

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