Advances in Luciferase Reporter Technologies

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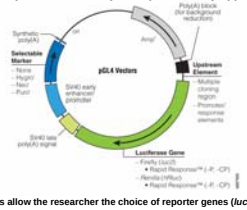
Abstract# 1193

1. Abstract

Luciferase reporter assays are critical tools for the cancer researcher because they are well suited for quantifying important cellular processes including proliferation, differentiation, and death. This is due to their wide dynamic range, low endogenous activity, and ease of use. In particular, luciferase reporter assays have been crucial tools in elucidating transcriptional regulatory mechanisms that control cell many aspects of cell physiology and function. Recent improvements within the pGL4 family of luciferase reporter vectors have further improved the utility of these vectors to meet the demands researchers in both academic and pharmaceutical settings. Our efforts to improve the luciferase reporter gene have enhanced both the dynamic range and the temporal response of luciferase reporter assays. We codon optimized the *luc2* and *hRluc* reporter gene open reading frames to facilitate more efficient expression leading to higher levels of luciferase protein. Comparison of several different cell lines transfected with *luc2* versus *luc+* reporter vectors validates that *luc2* transfected cells have significantly higher light output than *luc+* transfected cells. To enable more rapid assay kinetics and reducing assay time, we incorporated protein destabilization domains into the luciferase protein sequence. The combination of these improvements results in a reporter gene that allows high luciferase protein expression that closely mirrors the transcriptional status of the luciferase gene. In addition the pGL4 vector backbone has been optimized to significantly reduce the number of potential consensus transcription factor binding sites and lower uninduced levels of the luciferase reporter. These improvements have been incorporated into our GloResponse™ reporter cell lines to generate high quality stable reporter cell lines that facilitate study of the cAMP and NFAT signaling pathways in HEK293 cells.

2. Features of the pGL4 Vector family

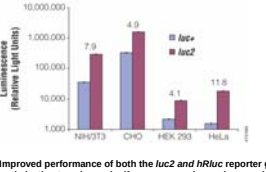
Customize your vector to fit your reporter assay application



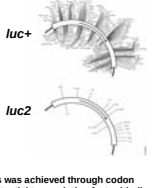
The pGL4 series of vectors allow the researcher the choice of reporter genes (*luc2*/*hRluc*/Rapid Response™), reporter gene regulatory elements (promoters/response elements) and selectable markers (Hygro'/Neo'/Puro'/or none) to best suit their reporter assay vector needs.

3. pGL4 Vectors use optimized luciferase reporter genes for enhanced expression and lower background

Codon optimization of *luc2* gene provides enhanced luciferase expression



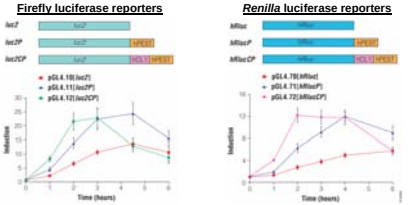
Removal of transcription factor binding sites within *luc2* gene



Improved performance of both the *luc2* and *hRluc* reporter genes was achieved through codon optimization to enhance luciferase expression and removal of potential transcription factor binding sites within the reporter gene sequence. A comparison of luciferase expression from cells transfected with reporter vectors using either *luc+* or *luc2* in the identical vector backbone demonstrate higher luciferase expression from the *luc2* gene.

4. Rapid Response™ pGL4 Vectors: Improved assay dynamics via destabilized luciferase reporters

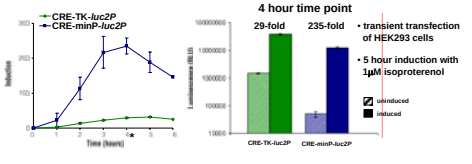
Firefly luciferase reporters | ***Renilla* luciferase reporters**



Rapid Response™ firefly and *Renilla* reporter genes contain either a PEST(*luc2P*/*hRlucP*) or both PEST and hCL1 (*luc2CP*/*hRlucCP*) codon-optimized protein degradation sequences to increase reporter gene turn-over. Comparison of luciferase induction after forskolin treatment of cells transfected with vectors expressing the Rapid-Response™ reporter genes under the control of a cAMP response element (CRE) demonstrate the increased response kinetics of the Rapid Response™ reporter genes.

5. Minimal Promoter pGL4 Vectors facilitate sensitive and robust measurement of response element activity

Lower uninduced luciferase expression in pGL4 Minimal Promoter Vectors leads to broader assay dynamic range

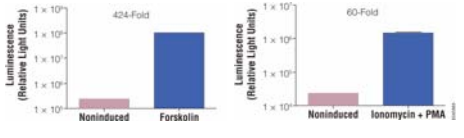


- transient transfection of HEK293 cells
- 5 hour induction with 1µM isoproterenol

A time course analysis of CRE induction of *luc2P* in vectors using either the minimal promoter (minP) or the HSV thymidine kinase (TK) promoter demonstrates a larger induction from the CRE-minimal promoter-*luc2P* vector. This higher induction is due to a lower uninduced level of luciferase expression.

6. CRE and NFAT-RE-containing pGL4 vectors are high quality, turn-key solutions for GPCR activity studies

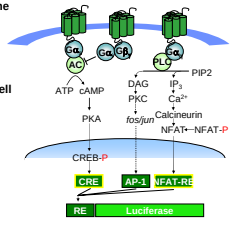
pGL4.29[*luc2P*/CRE/Hygro] | **pGL4.30[*luc2P*/NFAT-RE/Hygro]**



The pGL4.29[*luc2P*/CRE/Hygro and] pGL4.30[*luc2P*/NFAT-RE/Hygro] Vectors contains the *luc2P* reporter gene under the control of a CRE/minimal promoter element or NFAT-RE/minimal promoter element respectively. Transient transfection of these vectors into HEK 293 cells and induction with either100uM forskolin (pGL4.29) for 4 hours or 10ng/ml PMA/1µM ionomycin (pGL4.30) for 17 hours demonstrate robust luciferase induction (424 and 60-fold respectively). These vectors also contain the hygro' resistance gene for selection of stable cell lines.

7. GloResponse™ cell lines: The performance and advantages of pGL4 vectors in stable cell lines

- GloResponse™-CRE-*luc2P* HEK293 cell line responds to signaling through Ga_s or Ga_i-linked cAMP signaling pathway.
- GloResponse™ NFAT-RE-*luc2P* HEK293 cell line responds to signaling through Ga_q-linked IP_3/Ca2+ signaling pathway.
- Both cell lines use the Rapid Response™ *luc2P* reporter for rapid reporter assay kinetics.



8. pF9A CMV h*Rluc-neo* Flexi® Vector provides target expression and Renilla luciferase internal control

- The pF9A Vector enables generation of double stable cell lines expressing the target of interest plus the normalization Renilla-neo fusion control protein.
- pF9A vector utilizes Flexi® cloning technology for convenient cloning of target cDNA.
- Dual luciferase measurements provides experimental data from firefly luciferase signal and internal control data from Renilla luciferase.
- Renilla luciferase activity measurements enable well-to-well normalization and identification of suspect results from cytotoxicity or compound interference.

GloResponse™ *luc2P* HEK293 Cell Lines

pF9A-CMV-*hRluc-neo* Flexi® Vector

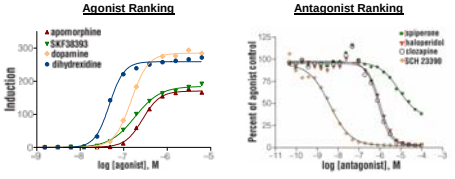
9. GloResponse™ Cell Lines are well suited for HTS applications

High Z' agonist reporter assay values from double stable cell lines generated from GloResponse™ Cell Lines using pF9A CMV *hRluc-neo* Flexi® Vector

GloResponse™ cell line	pF9A-expressed receptor	Fold Induction	Z'	Agonist
GloResponse™-CRE	Dopamine D1(DRD1)	56	0.77	SKF38393
GloResponse™-CRE	Muscarinic 3 (M3R)	18	0.70	carbachol
GloResponse™-NFAT-RE	Muscarinic 1 (M1R)	23	0.67	muscarine chloride
GloResponse™-NFAT-RE	Muscarinic 3 (M3R)	20	0.73	muscarine chloride

10. GloResponse™-CRE/DRD1 double stable cell line agonist and antagonist analysis

Agonist Ranking | **Antagonist Ranking**



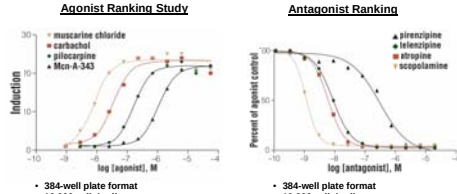
- 96-well plate format
- 10,000 cells/well
- 4 hour stimulation

- 96-well plate format
- 10,000 cells/well
- 4 hour stimulation
- 1µM dopamine as agonist

A GloResponse™-CRE/DRD1 double stable cell was generated by stable transfection of the GloResponse™-CRE Cell Line with a pF9A CMV *hRluc-neo* Flexi® Vector expressing DRD1 and subsequent clonal selection. This double stable cell line is well suited for DRD1 activity assays, including agonist and antagonist ranking studies as shown above.

11. GloResponse™-NFAT-RE/M3R double stable cell line agonist and antagonist analysis

Agonist Ranking Study | **Antagonist Ranking**



- 384-well plate format
- 10,000 cells/well
- 6 hour stimulation

- 384-well plate format
- 10,000 cells/well
- 4 hour stimulation
- 30nM muscarine chloride as agonist

A GloResponse™-NFAT-RE/M3R double stable cell line was generated by stable transfection of the GloResponse™-NFAT-RE Cell Line with a pF9A CMV *hRluc-neo* Flexi® Vector expressing M3R and subsequent clonal selection. This double stable cell line is well suited for M3R activity assays, including agonist and antagonist ranking studies as shown above.

12. Summary of pGL4 Vector and GloResponse™ luciferase reporter technology improvements

- Enhanced luciferase expression through codon optimization of *luc2* and *hRluc* genes
- Improved reporter assay kinetics via Rapid Response™ destabilized luciferase reporters
- Enhanced reporter assay sensitivity and dynamic range from Minimal Promoter pGL4 Vectors
- Robust turnkey reporter vectors for studying GPCR signaling pathways via pGL4.29[*luc2P*/CRE/Hygro] and pGL4.30[*luc2P*/NFAT-RE/Hygro] Vectors
- Incorporation of the performance and advantages of pGL4 vectors into GloResponse™ Cell Lines for high quality GPCR screening applications
- Utilization of Renilla-neo fusion protein as a normalization control when making double stable cell lines from GloResponse™ Cell lines with the pF9A CMV *hRluc-neo* Flexi® Vector
- Additional information available at the Promega Exhibitor Booth #518