SRE reporter assay for MAPK/ERK signaling via RTK and GPCR

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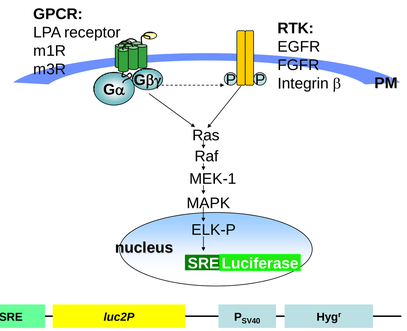
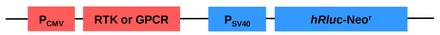
Abstract

Bioluminescent reporter assays are uniquely suited for high throughput screening due to their inherently high sensitivity, wide dynamic range and low susceptibility to compound interference. Here, we have incorporated the technical advancement of luciferase reporter genes and vectors to build an improved serum response element(SRE) luciferase construct for MAPK/ERK signaling pathway. Large dynamic range of SRE luciferase reporter assay allows efficient screening for RTK inhibitors for receptor tyrosine kinase pathway and also GPCR modulators for GPCR pathway. The dual-luciferase assay system, whereas the second plasmid expresses the target receptor of interest and Renilla luciferase as internal control, improves data quality by providing simultaneous monitoring of nonspecific effects such as cytotoxicity.

SRE Luciferase Reporter Assay for MAPK Signaling

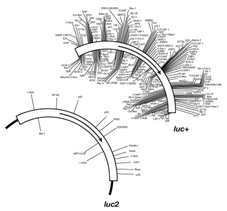


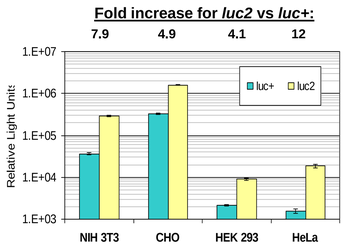
SRE-*luc2P* reporter responds to RTK or GPCR-linked MAPK/ERK signaling pathway.



pF9A Vector expresses the RTK or GPCR of interest and Renilla-neo fusion control protein for normalization.

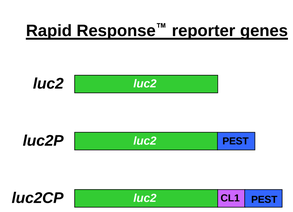
Codon Optimization Increases Expression of Luciferase Reporter Gene (luc2)

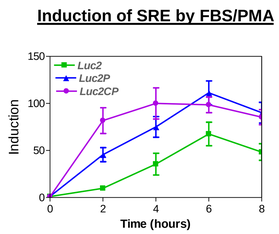




The coding sequence for the firefly luciferase was optimized for improved expression in mammalian cells. Consensus regulatory sequences such as transcription factor binding sites were also removed to reduce the risk of off-target expression.

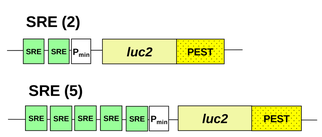
Rapid Response™ Luciferase Enhances Reporter Dynamics

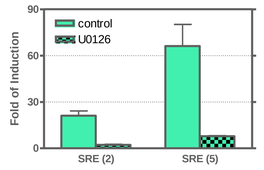




Versions of the *luc2* gene containing protein degradation sequences were used to enhance reporter dynamics. HEK293 cells transiently expressing SRE-*luc2*, SRE-*luc2P* or SRE-*luc2CP* were induced with 20%FBS plus 10ng/ml PMA. Firefly luciferase activity was quantified every other hour for 8 hours after induction using Luciferase Assay System.

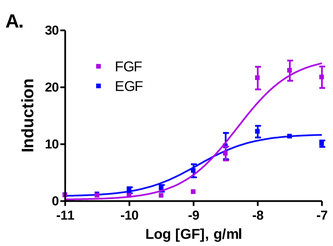
Optimized Reporter Repeats Further Improves Reporter Dynamics

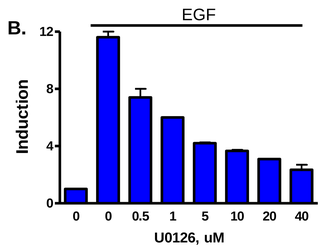




HEK293 cells were transiently transfected with SRE(2)-*luc2P* or SRE(5)-*luc2P* with Renilla luciferase in 96-well plates. Cells were pretreated with/without U0126 (MEK inhibitor) for 30 min, then induced with 10ng/ml PMA for SRE reporter assay. Firefly luciferase activity was quantified six hours after induction using the Dual-Glo™ Assay System.

Measure MAPK Signal via RTK





HEK293 cells were transiently transfected with SRE reporter vector SRE(5)-*luc2P* with Renilla luciferase in 96-well plates. Cells were induced with 1:3 serial dilutions of FGF or EGF (A), or pretreated with U0126 (MEK inhibitor) as indicated, then induced with 10ng/ml EGF (B). Firefly luciferase activity was quantified six hours after induction using the Dual-Glo™ Assay System.

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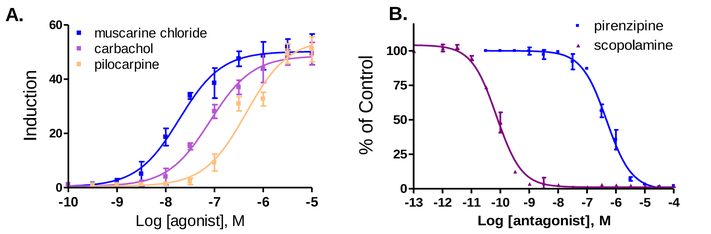
Measure MAPK Signaling via GPCR



HEK293 cells were transiently transfected with SRE-*luc2P* and muscarinic receptor 3 (M3R)-Renilla fusion, and serum starved overnight. Twenty-four hours after transfection, firefly luciferase activity was measured after six hours induction with addition of 1:3 serial dilutions of agonists (A), or pretreatment with antagonists before induction with 1 µM carbachol (B) using the Dual-Glo™ Assay System in 96-well format. For agonist assay, fold Induction = induced Firefly RLU/uninduced Firefly RLU, normalized to *Renilla* RLU. For antagonist assay, % of Control=[Firefly RLU (antagonist+agonist)/Firefly RLU (agonist alone)] ×100

Summary of Fold change and EC_{50} Value of Various Receptor/Agonist Pairs

	receptor	inducer	Fold Induction	EC_{50} (M)
RTK	EGFR	EGF	11	3.5×10^{-9}
	FGFR	FGF	18	5.2×10^{-9}
GPCR	LPA receptor	LPA	17	2.8×10^{-7}
	M1R	carbachol	46	3.4×10^{-7}
	M3R	carbachol	60	4.5×10^{-8}

HEK293 cells transiently expressing SRE-*luc2P,* Renilla luciferase and various endogenous (EGFR, FGFR, LPA receptor) or exogenous receptors (M1R, M3R) were induced with agonists for six hours and analyzed in 96-well format. Fold Induction = induced Firefly RLU/uninduced Firefly RLU, normalized to *Renilla* RLU.

Summary

Together the SRE luciferase reporter assay provides an excellent tool in monitoring MAPK/ERK pathways via RTK and GPCR pathways.

- **The newest generation of synthetic luciferase gene (*luc2*) is 4-10 times brighter than native luciferase.**
- **Use of Rapid Response™ *luc2P* gene improves reporter dynamics and reduces assay time.**
- **Optimized RE repeats upstream of *luc2P* further improves the dynamic response and allows specific detection of signaling via MAPK/ERK pathway.**
- **Large dynamic range allows efficient screening for RTK inhibitors and GPCR modulators in drug discovery.**