

G-Protein Coupled Receptor Assay Using Dual Luciferase Stable Cell lines

Aileen Paguio, Frank Fan and Keith Wood

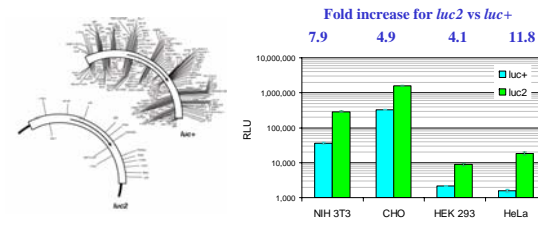
Promega Corporation, 2800 Woods Hollow Road, Madison WI 53711 Email: aileen.paguio@promega.com



Abstract

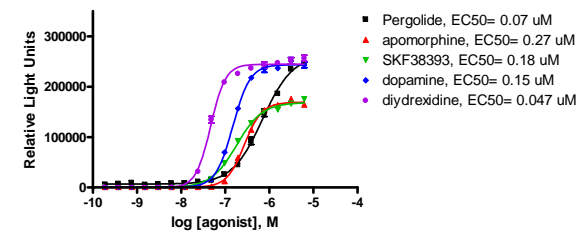
Bioluminescence-based technologies are great tools for high throughput drug screening because of their high sensitivity, wide dynamic range and less interference by the compounds. To reduce false positives generated by cytotoxicity of the screening compounds, it is desirable to include a control reporter (e.g. another luciferase) in the same assay system. However, such dual luciferase assay could not be simply configured by placing two luciferase genes on one vector due to cross interference between promoters and response elements (unpublished results). Therefore, we have developed a strategy of generating dual luciferase stable cell lines for GPCR assay. It involves two plasmids: one expressing firefly luciferase genes under the control of a response element (e.g. CRE) and a hygromycin selectable marker, and the other expressing target GPCR (e.g. dopamine receptor D1) and a Renilla luciferase-neomycin selectable marker fusion. In addition, destabilized luciferases were used to achieve more rapid signal response and shorter assay time. This helps to further the reduction of cytotoxicity caused by extensive contact time between the cells and the screening compounds. This dual-luciferase assay allows more rapid screening and improves data quality.

Improved Firefly Luciferase Reporter



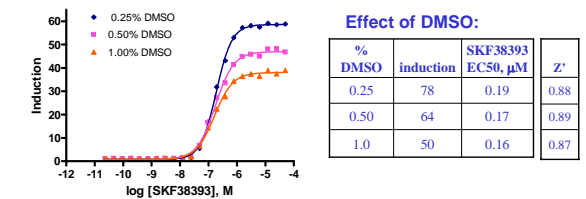
The coding sequence for the firefly luciferase was optimized for improved expression in mammalian cells. Consensus regulatory sequenced such as transcription factor binding sites were also removed to reduce the risk of off-target expression.

DRD1 Assay Allows Potency Ranking of Agonists



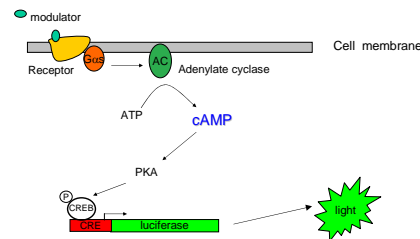
Double stable HEK293 cells expressing CRE-*luc2P* and dopamine receptor D1 was plated into a 96 well plate. Each dopamine agonist was serially diluted 1:2 and added to the well. Cells were harvested 4 hrs post treatment and assayed with Dual-Glo™ Assay System. The results are consistent with the ranking of these agonists by other methods.

DRD1 Assay Shows Solvent Tolerance

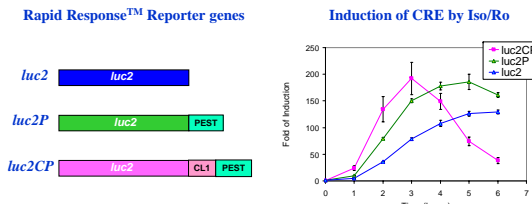


Double stable HEK293 cells expressing CRE-*luc2P* and dopamine receptor D1 were treated dopamine agonist 1 uM SKF38393 and 100uM Ro-20-1724 with different concentrations of DMSO. Cells were harvested 4 hrs post treatment and assayed with Dual-Glo™ Assay System. The addition of increased concentrations of DMSO has little effect on EC50 and Z' values.

Single Luciferase GPCR Assay

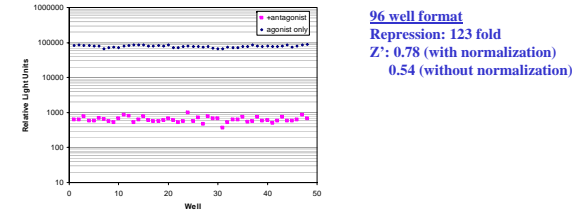


Rapid Response™ Luciferases Reduce the Time to Maximum Induction



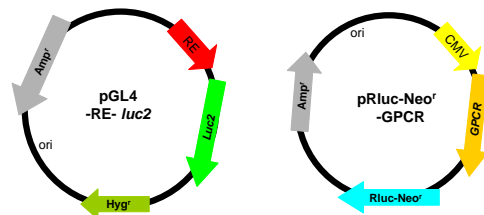
Versions of the *luc2* gene containing protein degradation sequences were used to reduce the time needed to assay maximum response (left). Stable cell lines containing these luciferase versions (*luc2*, *luc2P*, and *luc2CP*) revealed improved responsiveness to simulation of endogenous receptors (right). The cells, derived from HEK293, had the luciferase genes coupled to a CRE regulatory element. After activation of the cellular receptors with 1 uM isoproterenol/ 100 uM Ro-20-1724, samples were harvested every hour for quantifying luminescence.

High Quality DRD1 Antagonist Assay



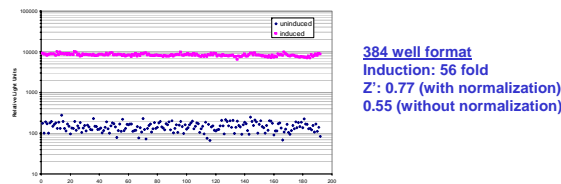
GPCR antagonist assay quality in 96 well format. Double stable HEK293 cells expressing CRE-*luc2P* and dopamine receptor D1 were treated 1 uM DRD1 antagonist, SCH23390 followed by 1 uM SKF38393, a DRD1 agonist and 100uM Ro-20-1724. Cells were harvested 4 hrs post treatment and assayed with Dual-Glo™ Assay System. Firefly luciferase activity was normalized to Renilla luciferase activity.

Dual Luciferase GPCR Assay: Two Readouts From One Sample



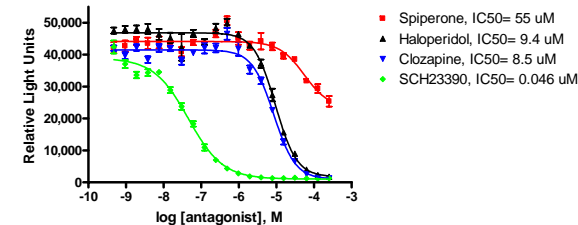
The dual luciferase assay employs two plasmids. One plasmid contains a response element upstream of the primary reporter gene firefly *luc2* and hygromycin selectable marker. The other plasmid expresses the GPCR of interest and a fusion of Renilla luciferase and neomycin selectable marker (Rluc-Neo').

High Quality DRD1 Agonist Assay



GPCR agonist assay quality in 384 well format. Double stable HEK293 cells expressing CRE-*luc2P* and dopamine receptor D1 were treated with 1 uM SKF38393, a DRD1 agonist and 100uM Ro-20-1724, using the BioMek FX liquid handling system. Cells were harvested 4 hrs post treatment and assayed with Dual-Glo™ Assay System, using the GENios Pro reader. Firefly luciferase activity was normalized to Renilla luciferase activity using the following formula where RLU= Relative Light Units: Normalized Firefly RLU= sample Firefly RLU/[sample hRluc RLU/average hRluc RLU]. Z' was calculated as 1 - [(3SDtreated + 3SDuntreated)/(Ave.treated - Ave.untreated)].

DRD1 Assay Allows Potency Ranking of Antagonists



Double stable HEK293 cells expressing CRE-*luc2P* and dopamine receptor D1 was plated into a 96 well plate. Each DRD1 antagonist was serially diluted 1:2 and added to well followed by the addition of 1 uM SKF38393/ 1 uM Ro-20-1724. Cells were harvested 4 hrs post treatment and assayed with Dual-Glo™ Assay System. The results are consistent with the ranking of these antagonist by other methods.

Summary

A GPCR assay using dual luciferase stable cell lines for GPCR assay with firefly luciferase as the primary reporter and a bi-functional fusion of *Renilla* luciferase and neomycin selectable marker is shown to have the following advantages:

- New generation of firefly luciferase (*Luc2*) and its Rapid Response versions provided brighter luminescence and improved responsiveness.
- A dual luciferase assay for dopamine receptor D1 showed good Z' values and dynamic range for both agonists and antagonists.
- Use of *Renilla* luciferase as an internal control improves data quality.
- Different agonists and antagonists can be ranked by EC50 and IC50. Results are consistent with other methods.
- Up to 1% DMSO has little effect on assay responsiveness and reproducibility.