IMPROVED RESPONSE RATIO FOR NF-κB INHIBITION ANALYSIS USING NEW LUCIFERASE REPORTER VECTOR (pGL4.32[LUC2P/NF-κB-RE/HYGRO])

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Promega has developed a new luciferase reporter vector for single- or dual-reporter gene measurements. Our experiments were performed in order to inhibit TNFα-induced nuclear transcription factor kappa B (NF-κB) activity by a natural product. Results with this new vector show improved reporter expression of firefly luciferase luminescence compared to a competitor vector. This new vector allows more accurate and precise measurement of NF-κB inhibition by bioactive compounds paralleled by an economically better consumption of the firefly luciferase vector.

Introduction

A fast and efficient study of internal cellular pathways was made possible by reporter genes; their application as a tool in molecular cell biology was first reviewed in 1988 (1). The Promega Dual-Glo™ Luciferase Assay System enables measurement of two independent reporter vectors in one sample, resulting in fast, reproducible and accurate determination of the luminescence signals of both vectors.

In our laboratory, this assay system was systematically used to measure the NF-κB inhibition activity of various biological compounds as a first, time-saving analytical step (2,3). NF-κB plays an important role in cell survival regulation (4).

NF-κB is an interesting target for anticancer research, since it is thought to be strongly involved in apoptotic events and inflammation; it is reported as the cause of cancer in 20 percent of all reported cases (5) and is involved in tumor promotion and progression. Moreover, this transcription factor is constitutively activated in a large number of different tumors (6). Consequently we chose to pursue the therapeutic strategy of inhibiting activation NF-κB, as a strategy for inducing apoptosis in different cancer cells. Inhibition of NF-κb has been described for several potential anticancer drugs currently in clinical or preclinical trials (7,8).

Recent research in our laboratory has focused on natural compounds extracted from sponges (9) as promising biological activities from secondary metabolites, which are produced by the host as a protection against predators (10,11). Here, we investigated the NF-κB inhibition activity of a newly isolated terpene of a sponge from the phylum Porifera using the Dual-Glo™ Luciferase Assay System. We compare the results of the new luciferase vector, pGL4.32 (*luc2P*/NF-κB-RE/Hygro), hereafter referred to as pGL4.32(NF-κB), to pNF-κB-Luc from Stratagene.

Materials and Methods

K562 cells were electroporated on a BioRad Gene Pulser® at a concentration of 1.5×10^7 cells/ml at 250 V and 500 µF.

Prior to transfection, 5 µg of firefly luciferase vector and 5 µg of *Renilla* plasmid (Promega) were added to each cuvette. Later in this study the amount of the firefly luciferase vector was reduced to 2.5 µg/transfection. After 24 hours of incubation at 37°C and 5% CO_2, the cells were centrifuged and a suspension of 10^6 cells/ml in RPMI medium (0.1% FCS, 1% antibiotic-antimycotic) was prepared.

A concentration range from 8.4 to 2.8 µM of the bioactive terpene was prepared. After 2 hours of incubation, TNFα was added at a concentration of 20 ng/ml, followed by an additional incubation period of 6 hours. The cell medium then was transferred to a 96-well plate (75 µl/well). Eight replicates were prepared for each concentration of terpene and for the negative and positive control solutions (TNFα at a concentration of 20 ng/ml).

After the incubations, 75 µl of Dual-Glo™ Luciferase Assay Reagent was added to each well, followed by a 10-minute reaction period at 22 °C. After measuring luminescence signal, 75 µl of Dual-Glo™ Stop & Glo® *Renilla* reagent was added to each well. After an additional 10 minutes at 22 °C, the luminescence was measured using a Berthold Orion Luminometer (integration period of 10 seconds).

The data shown in this article are the ratio of firefly to *Renilla* luminescence. All experiments were performed as a set of three independent replicates, and representative data are shown. Each dosage was tested eight times.

Results and Discussion

To distinguish the novel pGL4.32(NF-κB) from pNF-κB-Luc, independent measurements were performed with these two vectors and normalized against the same *Renilla* plasmid.

The terpene inhibited TNFα-induced NF-κB activation as expected. A higher induction of the luciferase luminescent activity was readily observed in the case of the newly developed luciferase vector, pGL4.32(NF-κB). In fact, the activation with TNFα in comparison to the control is 49-fold for pNF-κB-Luc and 227-fold with pGL4.32(NF-κB). This

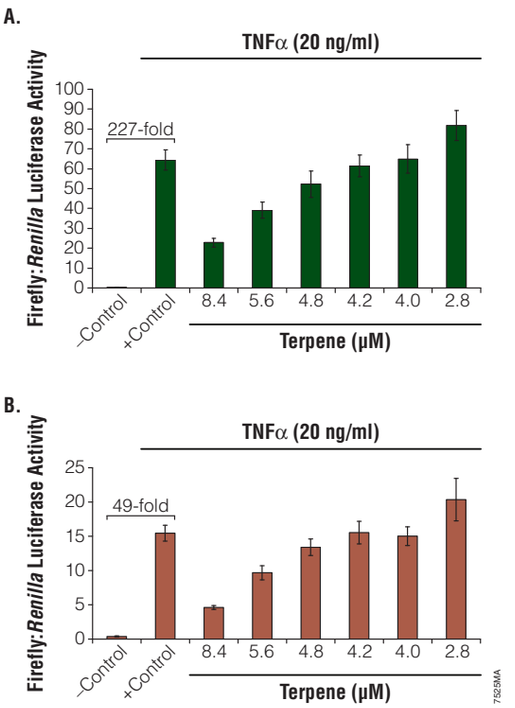

Figure 1. Characterization of K562 cells transfected with the newly developed luciferase vector, pGL4.32(NF-κB) (Promega) at 5 µg/transfection (Panel A) and pNF-κB-Luc, from Stratagene (Panel B). In both cases, the cells were co-transfected with the same *Renilla* plasmid, phRG-TK (Promega) at a concentration of 5 µg/transfection. See "Materials and Methods" for protocol. Panels A and B illustrate that the firefly luciferase encoded by the pGL4.32 (NF-κB) vector (Promega) is more strongly induced than that encoded by pNF-κB-Luc (Stratagene). The activation with TNFα in comparison to the control is 49-fold with the Stratagene vector and 227-fold with the Promega pGL4.32(NF-κB) Vector.

five-times higher induction could explain the better response linearity of the new luciferase plasmid. This better linearity (Figure 1) is an improvement for determining the fifty percent inhibitory concentration of bioactive compounds (IC_{50})

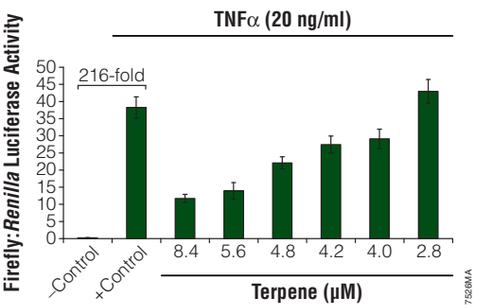

Figure 2. The amount of pGL4.32(NF-κB) plasmid used for electroporation can be reduced by 50% to 2.5 µg/transfection. At the lower concentration, linearity and quality of the measured relative luciferase activity were still suitable for ongoing analytical studies. Each measurement was assessed three times. Data are represented as mean +/– SD.

compared to pNF-κB-Luc from Stratagene; Figure 1, Panel B), so the number of necessary measurements can be effectively reduced with the new plasmid.

In a second set of measurements, the amount of added luciferase plasmid was reduced from 5 µg per electroporation to 2.5 µg (Figure 2). The quality of the response linearity of this experiment clearly indicated that a lower amount of luciferase vector can be used; subsequently the consumption of the luciferase plasmid can be lowered by 50%.

Summary

The Dual-Glo™ Luciferase Assay System is in frequent use in research; quick and accurate measurements are becoming more important in order to achieve higher efficiency and to increase the throughput number of samples. The development of the new luciferase reporter vector [pGL4.32(NF-κB)] contributes to these goals and can be considered an improved analytical tool in molecular cell biology.

References

1. Gould, S.J. and Subramani, S. (1988) *Anal. Biochem.* **175**, 5–13.
2. Morceau, F. *et al.* (2004) *Biochem. Pharmacol.* **67**, 1227–38.
3. Folmer, F. *et al.* (2008) *Biochem. Pharmacol.* **75**, 603–17.
4. Dolcet, X. *et al.* (2005) *Virchows Arch.* **446**, 475–82.
5. Coussens, L.M. and Werb, Z. (2002) *Nature* **420**, 860–7.
6. Jost, P.J. and Ruland, J. (2007) *Blood* **109**, 2700–7.
7. D'Incalci, M. *et al.* (2007) *Curr. Pharm. Des.* **13**, 2744–50.
8. Gilmore, T.D. *et al.* (2006) *Oncogene* **25**, 6887–99.
9. Blasius, R., Dicato, M. and Diederich, M. (2007) *Ann. N. Y. Acad. Sci.* **1095**, 377–87.
10. Rinehart, K. (1992) *Ciba Found Symp.* **171**, 236–54.
11. Sipkema, D. *et al.* (2005) *Mar. Biotechnol.* (NY) **7**, 142–62.

Protocols

Dual-Glo™ Luciferase Assay System Technical Manual, #TM058 (www.promega.com/tbs/tm058/tm058.html)

Ordering Information

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System	10 ml	E2920
pGL4.74[*hRluc*/TK] Vector	20 µg	E6921
pGL4.32(*luc2P*/NF-κB-RE/Hygro) Vector	20 µg	E8491

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