RapidReporter®

a brighter idea in luciferase assays

RapidReporter® Gaussia Luciferase Assay is the only reporter gene assay available that utilizes double destabilizing elements to degrade the luciferase protein AND mRNA. By dramatically reducing the background, the assay's response time and sensitivity are greatly improved.

RapidReporter advantages

- Double destabilizing elements to degrade both luciferase protein and mRNA
- Stronger fold induction reduces false positives
- Reduced background increases response time and sensitivity
- 2 formats – pRR High (best response to stimuli) or pRR-Low (stronger basal signal)
- Features *Gaussia* luciferase, which is brighter than firefly and *Renilla* luciferases
- Offered both as empty vectors and as pre-mades, which already contain widely studied promoters

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

RapidReporter® – double destabilization improves assay response

Active Motif's RapidReporter® is a patented* luciferase reporter gene assay that provides a faster, more pronounced response to stimulation and repression than other systems. RapidReporter vectors include destabilization elements for both the luciferase protein and its mRNA, so the assay yields more accurate kinetic and drug concentration-dependent responses. This vastly improves the monitoring of transcription factor activation and the detection of active compounds during high-throughput screening.

Because of their simplicity and versatility, reporter gene assays are an important tool for studying signal transduction pathways and gene expression. In such assays, a promoter, transcription factor-binding site or enhancer element is cloned into a vector upstream of a reporter gene, commonly luciferase. After transfection, the cells are treated to induce or repress transcription from the cloned promoter element. The assay uses the change in luciferase protein levels as a measure of transcriptional activity. Standard luciferase assays, however, are limited by the fact that basal activity of the cloned promoter results in accumulations of luciferase mRNA and protein. The slow clearance rate of these pre-existing reporter molecules substantially delays and dilutes the measurable response to stimulation. In the case of repression, this is because the reporter mRNA continues to produce new (and long-lived) reporter protein long after transcription has stopped. In the case of stimulation, this is because the short, rapid increase in luciferase in response to whatever stimulation is being tested has proportionally little impact on the high steady-state levels already present.

As a result, with standard reporter gene assays, transient & relatively minor effects are hidden and kinetic assays are inaccurate. This is because a large proportion of the reporter protein measured in standard assays is derived from transcription that took place before the test agent was even added. The use of a protein destabilizing element alone only partially solves this problem because new protein continues to be made from pre-existing mRNA. The combined use of BOTH protein- and mRNA-destabilizing elements, however, dramatically improves the clearance rate, providing superior responsiveness.

The RapidReporter advantage

The RapidReporter vectors utilize a non-secreted form of the extremely bright *Gaussia* luciferase gene, and are available as "empty" vectors with a multiple cloning site (MCS) for insertion of promoters/enhancers, or as pre-made vectors that contain a widely studied promoter such as NFκB, CREB (CRE) or GR. In the RapidReporter method, cells are plated and transiently transfected with the appropriate RapidReporter vector. After an overnight incubation, the stimulator/repressor of transcriptional activity is added for the appropriate time. The cells are lysed and flash luminescence is measured. All vectors are available separately or as a complete kit, which includes an EF1α promoter-driven positive control vector, as well as optimized Lysis & Assay Buffers and substrate.

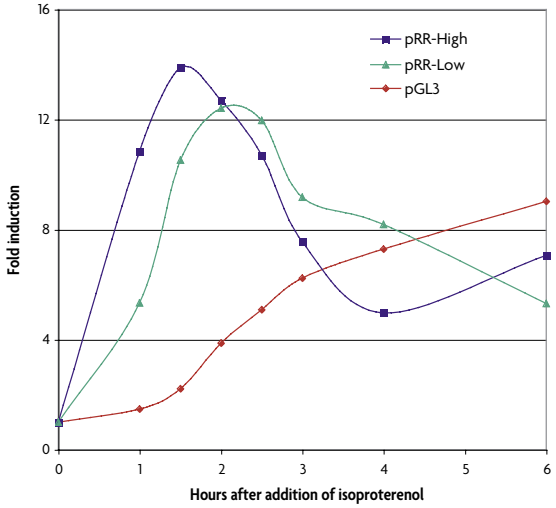

Figure 1: RapidReporter provides more accurate kinetics and a better fold induction than non-destabilized assays. 293 cells were transiently transfected with pRR-High-CRE, pRR-Low-CRE and pGL3 vector (non-destabilized) containing CRE (CREB response element) and plated onto 96-well plates. Twenty-four hours post-transfection, cells were stimulated with 4 µM isoproterenol. At the indicated time points, the cells were measured for *Gaussia* luciferase (pRR) and firefly luciferase activities (pGL3).

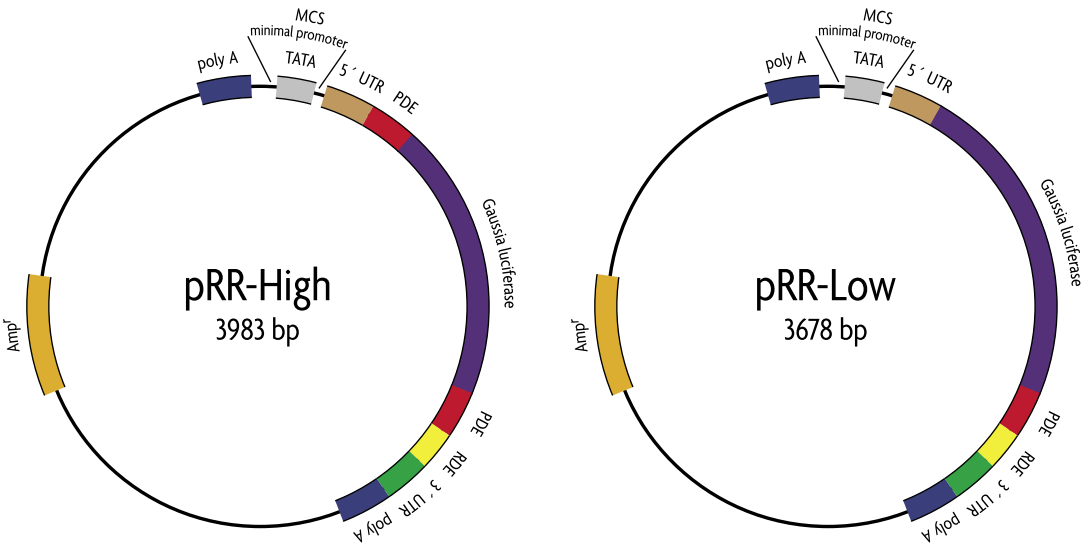

Figure 2: The RapidReporter pRR-High and pRR-Low vectors. The pRR-High plasmid contains two strong protein-destabilizing elements (PDE), while pRR-Low contains only one PDE. Each vector also contains an RNA-destabilizing element (RDE); the RDE in the pRR-High vector is strong while the RDE in the pRR-Low vector is weaker. The vectors also contain an intron for improved expression by enhancing RNA processing, and a synthetic optimized poly A signal to enhance luciferase expression.

Your choice of stringency

RapidReporter vectors are offered in two different stringencies, pRR-High and pRR-Low (Figure 2). The pRR-High vector provides the lowest background possible because it contains more and stronger destabilization elements, thereby providing maximum responsiveness. The fold induction of stimulated vs. non-stimulated samples is highest using pRR-High whereas the basal level will be lowest. This makes pRR-High vectors ideal in cases where your stimulation will only produce a weak effect. In contrast, the pRR-Low vector contains fewer and weaker destabilization elements. While this lowers the level of fold-induction observed, it enables weaker basal signals to be detected. pRR-Low vectors are ideal for use when a low signal strength is expected, such as with cells that transfect at a low efficiency or when testing a promoter with weak activity in the cells of interest. Regardless of the RapidReporter vector used, the fold induction observed will be higher than with other luciferase reporter systems such as firefly (Figure 1) and *Renilla* (data not shown).

Increased sensitivity to changes in transcription

RapidReporter vectors use double destabilizing elements to provide faster and more pronounced responses to drug effects than any other reporter gene system. This results in more accurate kinetic and drug concentration-dependent responses. For example, upon stimulation NFκB is released from IκB in the cytosol and translocates to the nucleus. However, it has been shown that NFκB translocation is not a single event.[1,2] Because of the quick responsiveness of RapidReporter, NFκB's oscillation from the cytosol to the nucleus upon activation is observed (Figure 3). Due to the long half life of the reporter protein in non-destabilized reporter systems such as pGL3 (firefly), this natural process is not detected.

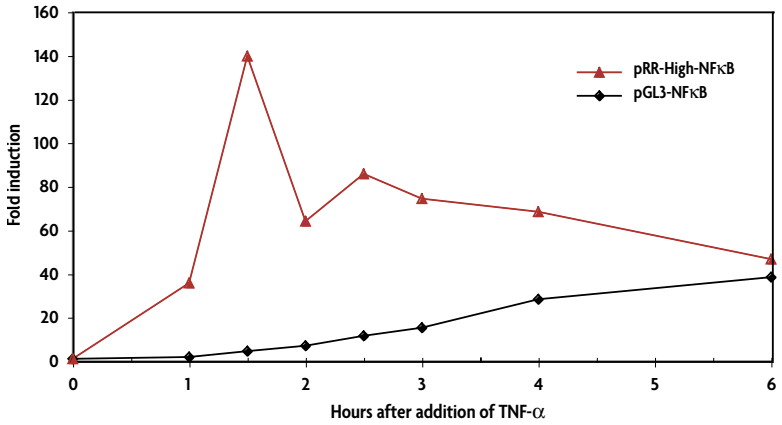

Figure 3: RapidReporter "unmasks" hidden effects.
HeLa cells were transiently transfected with pRR-High-NFκB and pGL3 vector containing NFκB and plated in 96-well plates. Twenty-four hours post-transfection, cells were stimulated with 10 ng/ml TNF-α. At the indicated time points, the cells were measured for *Gaussia* Luciferase (pRR) and firefly luciferase (pGL3). Because RapidReporter has double destabilizing elements, NFκB's natural oscillation from the cytosol to the nucleus during activation is observed.

Optimized buffers and substrates ensure success

Active Motif's Gaussia Luciferase Assay Kit contains proprietary Lysis and Assay Buffers and a *Gaussia* Substrate, all of which have been optimized for use with the *Gaussia* luciferase-fusion proteins expressed by the RapidReporter vectors. Use of these reagents enables you to take advantage of the enhanced sensitivity of the pRR-High and -Low plasmids. Because the *Gaussia* luciferase gene encoded by pRR-High and -Low has been modified for intracellular expression, it is not suitable for use with standard *Gaussia* assay kits or assay kits designed for use with other luciferases (Figure 4). For your convenience the RapidReporter Gaussia Luciferase Assay Kits are available in both 100 and 1000 reaction formats.

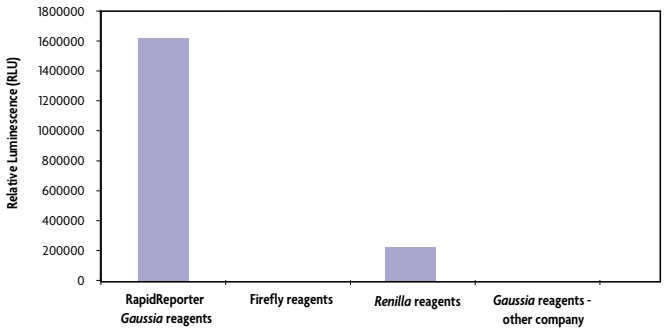

Figure 4: RapidReporter substrates and buffers work best.
RapidReporter pRR-Low was tested with substrates and lysis buffers for firefly, *Renilla* and another company's *Gaussia*. As depicted in the graph, RapidReporter reagents provide the best results with the RapidReporter vectors.

Pre-made vectors for transcription factors

For your convenience, Active Motif offers a number of pre-made vectors that already contain widely studied promoters. Currently, pre-made vectors are available for CREB (CRE), NFκB and GR, with more on the way. Each pre-made is available in the pRR-High vector (Figure 2) to maximize responsiveness to changes in transcription.

Ordering information

Product	Format	Catalog No.
RapidReporter® Gaussia Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter® pRR-High vector	10 µg	33003
RapidReporter® pRR-High Assay	100 rxns	33004
RapidReporter® pRR-Low vector	10 µg	33005
RapidReporter® pRR-Low Assay	100 rxns	33006
RapidReporter® pRR-High-CRE vector	10 µg	33007
RapidReporter® pRR-High-CRE Assay	100 rxns	33008
RapidReporter® pRR-High-NFκB vector	10 µg	33009
RapidReporter® pRR-High-NFκB Assay	100 rxns	33010
RapidReporter® pRR-High-GR vector	10 µg	33011
RapidReporter® pRR-High-GR Assay	100 rxns	33012

CONTENTS & STORAGE

The RapidReporter Gaussia Assays provide 100 or 1000 (96-well) reactions of 5X Lysis and 1X Assay Buffers and Gaussia Substrate. Vector alone kits provide 10 µg of either empty or pre-made vector. RapidReporter Assays sold with vector contain 10 µg of the appropriate vector, 100 reactions of 5X Lysis and 1X Assay Buffers and Gaussia Substrate, as well as a positive control vector driven by the EF1α promoter. Store at -20°C, see manual for details. All reagents are guaranteed stable for 6 months when stored properly.

REFERENCES

1. Nelson, D. E. *et al.* (2004) *Science*. 306: 704-708.
2. Hoffmann. A *et al.* (2001) *Science*. 298: 1241-1245.