RapidReporter®

(version B1)

RapidReporter® Gaussia Luciferase Assay, 100 reactions (Catalog No. 33001)
RapidReporter® Gaussia Luciferase Assay, 1000 reactions (Catalog No. 33002)
RapidReporter® pRR-High Vector (Catalog No. 33003)
RapidReporter® pRR-High Assay (Catalog No. 33004)
RapidReporter® pRR-Low Vector (Catalog No. 33005)
RapidReporter® pRR-Low Assay (Catalog No. 33006)
Pre-made RapidReporter® Vectors and Assays (Catalog Nos. 33007-33020)

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Overview

Active Motif's patented RapidReporter® is the only reporter gene assay available that utilizes both mRNA and protein destabilizing elements in the sequence of the reporter vector. This causes the cell to rapidly degrade both the luciferase protein and its mRNA, so background caused by basal activity of the promoter being tested is greatly reduced. Lowering the background increases both the magnitude of the response that can be measured after stimulation or repression, and the speed in which the assay can measure changes in transcription (both increases and decreases). Thus, RapidReporter is more sensitive and responsive, so you can detect smaller changes and get more accurate real-time measurement of the transcriptional activity of your promoter than is possible using non-destabilized reporter gene assays, or those that destabilize the protein only (Figure 1, page 4).

RapidReporter vectors utilize a non-secreted form of the extremely bright *Gaussia* luciferase gene, and are available as an "empty" vector with a multiple cloning site (MCS) for insertion of promoters/enhancers, or as "pre-made" vectors that contain a widely studied promoter. Vectors are available separately or as a complete assay, which also include an EF1α promoter-driven positive control vector, as well as all of the optimized buffers and substrate required to measure activation of the reporter. The buffers and substrate are also available separately.

product	format	catalog no.
RapidReporter® Gaussia Luciferase Assay	100 rxns	33001
RapidReporter® Gaussia Luciferase Assay	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 Gaussia Luciferase Assays (Cat. Nos. 33001 & 33002) contain sufficient 5X Gaussia Lysis Buffer, Gaussia Substrate and 1X Gaussia Assay Buffer to perform either 100 or 1000 reactions, where a reaction is defined as a single well of a 96-well plate.

RapidReporter vectors contain 10 µg of vector only.

RapidReporter Assays contain 10 µg of the chosen vector, 10 µg of EF1α positive control vector, and sufficient 5X Gaussia Lysis Buffer, Gaussia Substrate and 1X Gaussia Assay Buffer to perform 100 reactions to measure Gaussia luciferase in a single well of a 96-well plate.

Active Motif also offers a variety of premade RapidReporter vectors that already contain widely studied promoters. Please give us a call or visit our website for up-to-date product information.

Introduction

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 that occurs 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 or relatively minor effects are hidden and kinetic assays are inaccurate.

In effect, 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. However, the combined use of BOTH protein- and mRNA-destabilizing elements dramatically improves the clearance rate and provides superior responsiveness[1].

The basis of Active Motif's RapidReporter technology lies in the use of both protein- and RNA-destabilizing elements to improve the responsiveness to changes in transcription. The benefit applies to both increases and decreases in transcription and includes both an enhanced magnitude of response and a faster (more real-time) response rate. The greater the strength of the destabilizing elements, the greater the improvement in responsiveness. However, stronger destabilizing elements also have a larger effect on reducing reporter activity; depending on the reporter type, your application, and the cell type & promoter being tested, this may or may not be problematic.

To overcome this potential limitation, Active Motif's RapidReporter system utilizes a modified form of Gaussia luciferase, the most sensitive luciferase known. In addition, all RapidReporter vectors have been extensively optimized to enhance expression levels (*e.g.* via improved RNA processing and translational efficiency). These modifications, together with the enhanced sensitivity of Gaussia luciferase and our associated assay reagents, provide a signal strength greatly exceeding that of firefly luciferase, even non-destabilized firefly luciferase.

Other tips on maximizing expression are contained in the Troubleshooting Section, page 19.

Note: It is important to note that the enhanced sensitivity of pRR-High and -Low plasmids requires their use in combination with Active Motif's Gaussia Luciferase Assay Kit, which has been specifically optimized for the Gaussia luciferase-fusion proteins expressed by these vectors. Importantly, the Gaussia luciferase gene encoded by pRR-High and -Low vectors has been modified for *intracellular* expression and is thus not suitable for use with generic Gaussia assay kits or assay kits designed for other luciferases. Wild-type Gaussia luciferase is a secreted protein and should not be confused with the intracellular Gaussia expressed by pRR vectors. It is therefore important to run your assays using only Active Motif's Gaussia Luciferase Assay Kit.

Choosing the Right Reporter Vector

Active Motif has developed two plasmids with different levels of RNA and protein destabilization. Our pRR-High series of reporter plasmids contain more and stronger destabilizing elements, resulting in maximal responsiveness. They are ideal for detecting small or transient changes that would otherwise be hidden. Our pRR-Low series of reporter plasmids contain fewer and weaker destabilizing elements; therefore, they provide higher steady-state reporter levels than those obtained with pRR-High. The responsiveness is still considerably faster and stronger than with any non-RapidReporter vector, but is less than that seen with pRR-High plasmids.

We recommend testing both reporter types in each new application. However, as a general guide to choosing the best vector for your purposes, consider the following:

- Is the activity of the pathway being measured very low in your cells of interest?
- Are your cells difficult to transfect?
- Do you need to perform glow reactions rather than taking full advantage of the exceptionally high flash signal?

If the answer to 2 or more of these questions is "yes," then it may be preferable for you to use pRR-Low or, alternatively, to try both reporter vectors.

Whereas the stronger destabilizing elements of pRR-High result in lower background and stimulated signals than with pRR-Low, the fold induction observed with pRR-High, which is what most experiments are designed to show, is greater with pRR-High than with pRR-Low. In addition, pRR-High responds to changes in transcription faster (Figure 1, page 4). With pRR-Low, however, signal strength is typically 8-10 fold higher.

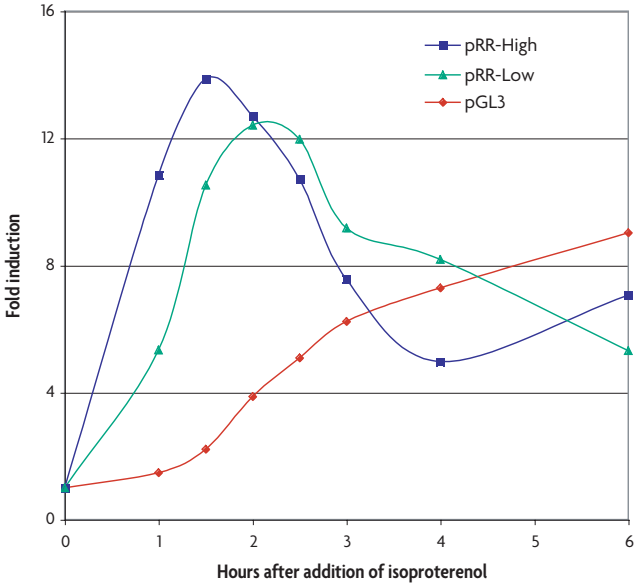

Figure 1: Induction with isoproterenol.
293 cells were transiently transfected with pRR-High-CRE, pRR-Low-CRE and a pGL3 vector containing CRE (CREB Response Element) and plated onto 96-well plates. Twenty-four hours post-transfection, cells were stimulated with 4 µM isoproterenol. At the indicated time points, the media was removed, the cells were lysed and then measured for Gaussia luciferase (pRR-High-CRE & pRR-Low-CRE) and firefly luciferase activity (pGL3-CRE).

Features of pRR-Low and pRR-High

PDE: Protein-destabilizing element. pRR-High contains 2 strong PDEs; pRR-Low has lower destabilization because it contains only 1 copy of a weaker PDE.

RDE: RNA-destabilizing element dramatically enhances responsiveness. Each vector contains one RDE, but the RDE in pRR-High is stronger than the one in pRR-Low; this contributes to the greater destabilization of pRR-High.

Intron: Improves expression by enhancing RNA processing.

Reporter: Gaussia luciferase.

poly A: Synthetic optimized poly A signal of reporter gene; enhances expression.

amp^r: Ampicillin resistance gene for plasmid amplification.

poly A: Synthetic upstream poly A signal reduces background leakiness.

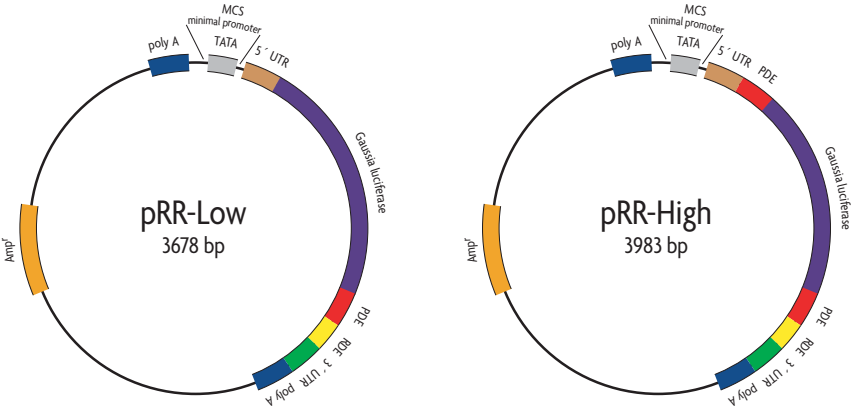

Figure 2: RapidReporter Plasmid Maps – pRR-Low & pRR-High.

pRR-Low Vector Sequence Reference Points (3678 bases)

Promoter	(none)
Enhancer	(none)
Multiple cloning site (MCS)	1-95
Gaussia luciferase gene (luc+)	317-1266
ColE1-derived plasmid replication origin	1751
β-lactamase gene (Amp^r)	2513-3372
Synthetic (upstream) poly(A) signal	3525-3678
pRR-REV: 5´-GTTCCCTGCTCTCTGTCG-3´	288-305
pRR-FWD: 5´-TCATTACATCTGTGTGTT-3´	3543-3560

pRR-High Vector Sequence Reference Points (3983 bases)

Promoter	(none)
Enhancer	(none)
Multiple cloning site (MCS)	1-95
Gaussia luciferase gene (luc+)	317-1530
ColE1-derived plasmid replication origin	2056
β-lactamase gene (Amp^r)	2818-3678
Synthetic (upstream) poly(A) signal	3830-3983
pRR-REV: 5´-GTTCCCTGCTCTCTGTCG-3´	288-305
pRR-FWD: 5´-TCATTACATCTGTGTGTT-3´	3848-3865

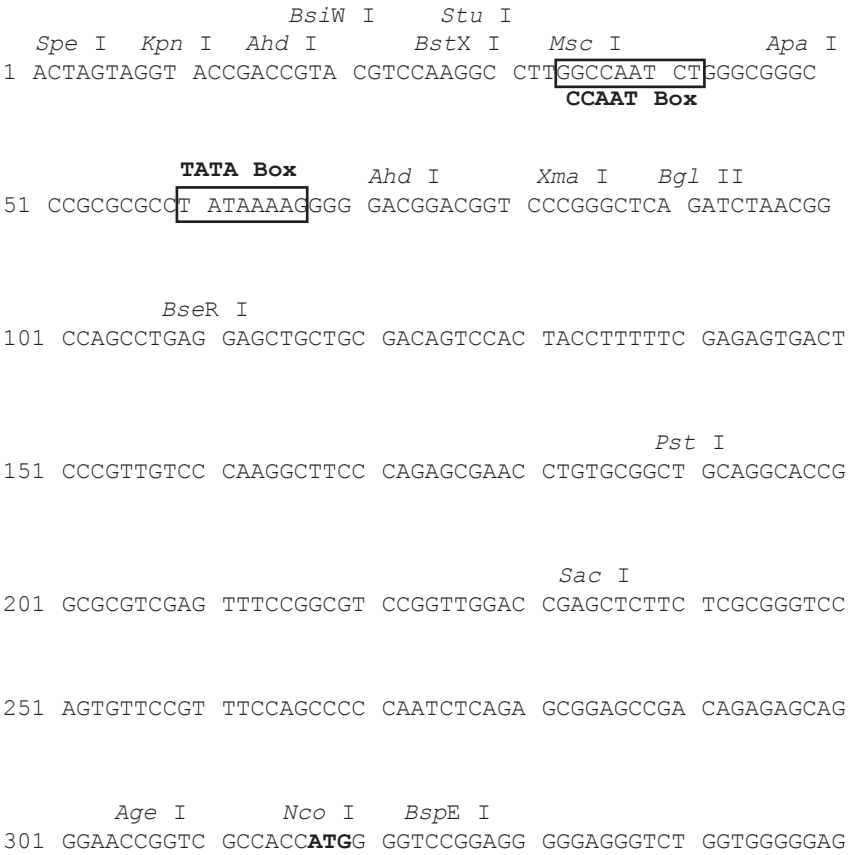

Figure 3: MCS/minimal promoter (promoterless versions only).
The RapidReporter vectors, pRR-Low and pRR-High, contain a multiple cloning site designed to offer maximum flexibility for inserting 1 or more copies of a single transcription factor-binding site, complex enhancers, an entire promoter, or an entire promoter plus its 5´ UTR.

pRR-Low Restriction Enzyme Sites (MCS)

3678 bp

Enzyme	No. of cuts	Position	Recognition Site
Age I	1	304	A▼CCGGT
Apa I	1	51	GGGCC▼C
Avr II	1	2440	C▼CTAGG
*Bam*H I	1	622	G▼GATCC
Bgl II	1	90	A▼GATCT
*Bsi*W I	1	17	C▼GTACG
*Bsp*E I	1	323	T▼CCGGA
*Bss*H II	1	53	G▼CGCGC
*Bst*X I	1	31	CCAnnnnn▼nTGG
*Eco*R I	1	1285	G▼AATTC
*Eco*R V	None	–	GAT▼ATC
Hind III	1	1138	A▼AGCTT
Hpa I	1	1390	GTT▼AAC
Kpn I / *Acc*65 I	1	12	GGTAC▼C
Mlu I	None	–	A▼CGCGT
Nco I	1	315	C▼CATGG
Nde I	None	–	CA▼TATG
Nhe I	1	1450	G▼CTAGC
Not I	1	3518	GC▼GGCCGC
Pvu II	None	–	CAG▼CTG
Sac I	1	236	GAGCT▼C
Sac II	1	714	CCGC▼GG
Sal I	1	1443	G▼TCGAC
Sma I	1	83	CCC▼GGG
Spe I	1	1	A▼CTAGT
Stu I	1	29	AGG▼CCT
Xba I	None	–	T▼CTAGA
Xho I	1	1319	C▼TCGAG
Xma I	1	81	C▼CCGGG

pRR-High Restriction Enzyme Sites (MCS)

3983 bp

Enzyme	No. of cuts	Position	Recognition Site
Age I	1	304	A▼CCGGT
Apa I	1	51	GGGCC▼C
Avr II	1	2745	C▼CTAGG
*Bam*H I	1	886	G▼GATCC
Bgl II	1	90	A▼GATCT
*Bsi*W I	1	17	C▼GTACG
*Bsp*E I	1	587	T▼CCGGA
*Bss*H II	1	53	G▼CGCGC
*Bst*X I	1	31	CCAnnnnn▼nTGG
*Eco*R I	1	1548	G▼AATTC
*Eco*R V	None	–	GAT▼ATC
Hind III	1	1402	A▼AGCTT
Hpa I	1	1695	GTT▼AAC
Kpn I / *Acc*65 I	1	12	GGTAC▼C
Mlu I	None	–	A▼CGCGT
Nco I	1	315	C▼CATGG
Nde I	None	–	CA▼TATG
Nhe I	1	1755	G▼CTAGC
Not I	1	3823	GC▼GGCCGC
Pvu II	1	466	CAG▼CTG
Sac I	1	236	GAGCT▼C
Sac II	2	541, 978	CCGC▼GG
Sal I	1	1748	G▼TCGAC
Sma I	1	83	CCC▼GGG
Spe I	1	1	A▼CTAGT
Stu I	1	29	AGG▼CCT
Xba I	None	–	T▼CTAGA
Xho I	1	1624	C▼TCGAG
Xma I	1	81	C▼CCGGG

Cloning Promoters and Transcription Factor-Binding Sites

The RapidReporter vectors, pRR-Low and pRR-High, contain a multiple cloning site designed to offer maximum flexibility for inserting either:

- 1 or more copies of a single transcription factor-binding site
- Complex enhancers
- An entire promoter
- An entire promoter plus its 5´ UTR

Useful notes on pRR-Low and pRR-High MCS restriction enzyme sites:

- *Stu* I and *Sma* I are blunt cutters.
- *Spe* I generates compatible cohesive ends with *Avr* II, *Nhe* I and *Xba* I.
- *Xma* I generates compatible cohesive ends with *Age* I and *Bsp*E I.
- *Bgl* II generates compatible cohesive ends with *Bam*H I.

Therefore, if you are planning to PCR a promoter that contains endogenous *Spe* I, *Xma* I or *Bgl* II sites, simply design your PCR primers with the alternative restriction enzyme sites that generates the appropriate compatible ends. See Figure 2 on page 5 for the sequence of all promoterless RapidReporter plasmids.

Inserting a Transcription Factor-Binding Site or Enhancer

- Use only the restriction enzyme sites that lie upstream of the TATA box.
- We recommend using *Spe* I or *Kpn* I as the 5´ cloning site.
- To maintain the CCAAT box, use *Bst*X I* as the 3´ cloning site.
- To remove the CCAAT box, use *Apa* I as the 3´ cloning site.

  Note: The presence of a CCAAT box may cause a slight increase in expression but a slight reduction in fold-inducibility.

* The *Bst*X I site in the MCS was engineered to provide compatible ends with *Apa* I. This allows the same DNA fragment (*e.g. Kpn* I-*Apa* I) to be inserted into pRR plasmids either with a CCAAT box (cut *Kpn* I / *Bst*X I) or without a CCAAT box (cut *Kpn* I / *Apa* I).

Where possible, we recommend trying both variants in your particular test system. Alternatively, maintain the CCAAT box in applications where a higher basal activity may provide better results (*e.g.* when using cells in which the basal activity of the inserted enhancer is expected to be extremely low) and remove the CCAAT box in applications where a reasonable level of basal activity is expected and maximal inducibility/repression is desired.

Inserting an Entire Promoter

- Use a 5´ restriction enzyme site that lies upstream of the CCAAT box and a 3´ restriction enzyme site that lies downstream of the TATA box.
- We recommend using either *Spe* I or *Kpn* I as the 5´ cloning site and either *Xma* I** or *Bgl* II as the 3´ cloning site.
- Alternatively, the *Ahd* I sites can be used to directly insert promoter fragments generated by PCR with polymerases (*e.g. Taq*) that generate a single 3´ A-overhang. The two *Ahd* I sites in pRR plasmids have been engineered to provide 3´ T-overhangs for this purpose.

Ideally the promoter fragment being inserted should contain as little as possible of its endogenous 5´ UTR but MUST contain its transcription initiation site. Some 5´ UTRs contain cis-acting inhibitors of translation, whereas the 5´ UTR of pRR plasmids significantly enhances translation.

** The *Xma* I site provides compatible ends with *Age* I. This allows a promoter-5´ UTR fragment (*e.g. Kpn* I-*Age* I) to be inserted into pRR plasmids either with the plasmid's 5´ UTR (cut *Kpn* I / *Xma* I) and/or without the plasmid's 5´ UTR (cut *Kpn* I / *Age* I).

Inserting an Entire Promoter plus its 5´ UTR

If you wish to maintain the endogenous 5´ UTR of your promoter of interest, use a 5´ restriction enzyme site that lies upstream of the CCAAT box and a 3´ restriction enzyme site that lies downstream of the TATA box. The following options are available:

- Use either *Xma* I or *Bgl* II as the 3´ cloning site (as above). This will create a fusion of the endogenous 5´ UTR and the pRR 5´ UTR. In our experience this option generally provides the highest expression levels.
- Use *Age* I as the 3´ cloning site to replace the pRR 5´ UTR with your 5´ UTR of interest; this will maintain the Kozak translation initiation sequence of pRR, which is immediately downstream of *Age* I.
- Use *Nco* I (CCATGG) as the 3´ cloning site. This will remove part of the Kozak sequence (GCCACCATGG), causing a decrease in expression unless replaced. However, *Nco* I allows insertion of endogenous 5´ UTR sequences to within 2 nt of the start codon.

Kit Components and Storage

Kit components can be stored at -20°C prior to first use. Then, we recommend storing each component at the temperature indicated in the tables below.

Gaussia Substrate: Store at ≤ -20°C. Protect from light. Packaged under Argon; do not open before use.

1X Gaussia Assay Buffer (minus substrate): Store at ≤ -20°C. Can be stored at 4°C for at least one month. Protect from light.

5X Lysis Buffer: Store at ≤ -20°C. Can be stored at 4°C for at least one month. Protect from light.

RapidReporter Vectors

Vector	Quantity	Storage / Stability
pRR-High vector	10 µg	-20°C for 6 months
pRR-Low vector	10 µg	-20°C for 6 months
Pre-made pRR-High fusion vectors	10 µg	-20°C for 6 months

RapidReporter Gaussia Luciferase Assay, 100 rxns*

Reagent	Quantity	Storage / Stability
5X Gaussia Lysis Buffer	400 µl	-20°C for 6 months
Gaussia Substrate	31.2 µl	-20°C for 6 months
1X Gaussia Assay Buffer	6 ml	-20°C for 6 months

RapidReporter Gaussia Luciferase Assay, 1000 rxns*

Reagent	Quantity	Storage / Stability
5X Gaussia Lysis Buffer	4 ml	-20°C for 6 months
Gaussia Substrate	312 µl	-20°C for 6 months
1X Gaussia Assay Buffer	60 ml	-20°C for 6 months

RapidReporter pRR-High Assay, 100 rxns*		
Reagent	**Quantity**	**Storage / Stability**
pRR-High vector	10 µg	-20°C for 6 months
pRR-High-EF1α vector	10 µg	-20°C for 6 months
5X Gaussia Lysis Buffer	400 µl	-20°C for 6 months
Gaussia Substrate	31.2 µl	-20°C for 6 months
1X Gaussia Assay Buffer	6 ml	-20°C for 6 months

RapidReporter pRR-Low Assay, 100 rxns*		
Reagent	**Quantity**	**Storage / Stability**
pRR-Low vector	10 µg	-20°C for 6 months
pRR-Low-EF1α vector	10 µg	-20°C for 6 months
5X Gaussia Lysis Buffer	400 µl	-20°C for 6 months
Gaussia Substrate	31.2 µl	-20°C for 6 months
1X Gaussia Assay Buffer	6 ml	-20°C for 6 months

Pre-made RapidReporter Assays, 100 rxns*		
Reagent	**Quantity**	**Storage / Stability**
pRR-High-fusion vector	10 µg	-20°C for 6 months
pRR-High-EF1α vector	10 µg	-20°C for 6 months
5X Gaussia Lysis Buffer	400 µl	-20°C for 6 months
Gaussia Substrate	31.2 µl	-20°C for 6 months
1X Gaussia Assay Buffer	6 ml	-20°C for 6 months

* Each reaction of RapidReporter is sufficient to measure Gaussia luciferase in a single well of a 96-well plate.

The RapidReporter vectors are for research use only. Not for use in diagnostic procedures.

Additional materials required

- TE (10 mM Tris, 1 mM EDTA pH 8.0)
- Bacterial culture medium and supplies
- Transfection grade plasmid DNA preparation kit, (*e.g.* QIAGEN Plasmid Maxi Kit, Catalog No. 12162).
- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- 100 mm tissue culture plates
- 96-well tissue culture plates (Costar 9102 strip-well plates are recommended)
- Transfection Reagent (*e.g.* FuGENE® 6 Transfection Reagent; Roche, Catalog No. 11814443001)
- Cell culture medium and standard cell culture supplies
- Reduced serum or serum-free medium (*e.g.* Opti-MEM® I Reduced Serum Medium, Invitrogen, Catalog No. 31985-062)
- Distilled water (dH_2O)
- Luminometer (*e.g.* Tecan, Research Triangle Park, NC)
- Hemacytometer

Component Preparation and Recommendations

Immediately prior to starting the assay, please prepare the following:

Preparation of Plasmid DNA Stock Solution

The pRR-High, pRR-Low, pRR-High-EF1α, pRR-Low-EF1α control, and all pre-made pRR vectors are supplied lyophilized as 10 µg aliquots. Resuspend the lyophilized DNA in 10 µl of TE in the provided vial (1 µg/µl). This Stock Solution can be stored at -20°C for 6 months. Use this Stock DNA to transform competent *E. coli* cells (*e.g.* DH5α). Purify plasmid DNA from LB cultures using a commercially available transfection grade plasmid DNA preparation kit, (*e.g.* QIAGEN Plasmid Maxi Kit, Cat No. 12162).

Preparation of 1X Gaussia Lysis Buffer

Prepare a sufficient quantity of 1X Lysis Buffer by adding 1 volume of 5X Lysis Buffer to 4 volumes of distilled water and mixing well. Protect Lysis Buffer from light. Close vial/bottle immediately after use. If a precipitate is noticed upon thawing of the 5X Lysis Buffer, pipet the solution up and down carefully to redissolve.

Preparation of Gaussia Assay Buffer with Substrate

Prepare the Gaussia Assay Buffer by adding 5 µl of Gaussia Substrate for every 1 ml of 1X Gaussia Assay Buffer required. Close the vial of Gaussia Substrate immediately after pipetting. For best results prepare Gaussia Assay Buffer with Substrate ~30 minutes before use. Storage of Assay Buffer with Substrate will result in a loss of activity over time. If a precipitate is noticed upon thawing of the 1X Gaussia Assay Buffer, pipet the solution up and down carefully to redissolve. Protect Gaussia Assay Buffer with Substrate from light using aluminum foil.

Note: Protect the Gaussia Substrate from light. The substrate is packaged under argon; do not open before use.

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

DAY 1: Plating Cells

Plate cells at 1.7 x 10^6 cells/100 mm plate.

Note: Plating this amount of cells should yield 50-80% confluence on the day of transfection. Low confluence is required to allow enough surface area for growth during the experiment period.

1. Carefully aspirate the media from a confluent 100 mm plate and briefly wash twice with 10 mL of PBS.
2. Add 1 ml trypsin and incubate for 5 minutes at 37°C in a humidified atmosphere containing 5% CO_2.
3. Resuspend the cells with 9 ml of cell growth media and transfer to a sterile 50 ml conical tube.
4. Count the cells using a hemacytometer.
5. Dilute the cells to the correct density, plate 10 ml on 100 mm plates and incubate overnight at 37°C in a humidified atmosphere containing 5% CO_2.

DAY 2: Transient Transfection

Perform a transient transfection of the cells. We recommend FuGENE® 6 Transfection Reagent (Roche, Catalog No. 1181444300). Follow the manufacturer's recommendations. Other commercially available transfection reagents are also suitable. The provided protocol is suitable for one 100 mm plate of cells.

1. For each 100 mm plate of cells to be transfected, prepare a diluted FuGENE 6 solution by adding 34 µl FuGENE 6 Reagent to 550 µl of Opti-MEM® I Reduced Serum Medium or serum-free culture media. Do not touch the sidewall of the microcentrifuge tube.
2. Tap to mix; incubate for 5 minutes at 37°C in a humidified atmosphere containing 5% CO_2.
3. Add approximately 5 µg of your pRR vector stock solution to the diluted FuGENE 6 mix.

   Note: Do not allow undiluted FuGENE 6 Reagent (in Step 1) to come into contact with plastic surfaces other than pipette tips. Once the FuGENE® 6 Reagent is diluted, it must be combined with the DNA within 45 minutes.

4. Tap to mix and incubate for 15 minutes at 37°C in a humidified atmosphere containing 5% CO_2 to allow DNA/transfection reagent complexes to form.
5. Ensure that the cells from Day 1 are the correct cell density.
6. Tap to mix the transfection mix again and add the entire mixture to the plate. Swirl and

return the plate to the 37°C incubator containing 5% CO_2 (there is no need to remove the media or wash the cells). Allow the cells to transfect for at least 4-5 hours before re-plating.

Replating on 96-well Plates

We recommend Costar 9102 strip-well plates.

1. Carefully aspirate the media from the 100 mm plate and wash 2X with 10 ml sterile PBS.

   Note: Add the PBS slowly to the side of the plate to minimize disturbing the cells.

2. Add 1 ml trypsin and incubate for 5 minutes at 37°C in a humidified atmosphere containing 5% CO_2.
3. Resuspend the trypsinized cells with 9 ml of cell growth media and transfer to a 50 ml conical tube for counting.
4. Count cells using a hemacytometer to determine cells per ml of media. Dilute cells with cell growth media to 30,000 cells per 190 µl of media (depending on the cell line used, optimal plating density will vary).
5. Transfer the transiently transfected cells to a sterile plastic reservoir and add 190 µl to each well using a multi-channel pipettor.
6. Return 96-well plates to the 37°C incubator (humidified atmosphere containing 5% CO_2) for overnight (at least 16 hours).

DAY 3: Stimulation/Repression of Transcription

1. Prepare a 20X stock of transcriptional stimulator/inhibitor in cell growth media.
2. Add the 20X stock to a sterile plastic reservoir.
3. Using a multi-channel pipettor add 10 µl of 20X stock stimulator/inhibitor to each well designated for stimulation.

   Note: Control wells do not receive any treatment or may be treated with the appropriate carrier control.

4. Return the plate to the 37°C incubator for the amount of time required for treatment.

Lysis and Measurement

Use the 1X Lysis Buffer and Assay Buffer **with Substrate** prepared as described on page 15.

1. Remove media from 96-well culture plate and add 20 µl 1X Lysis Buffer to each well.
2. Incubate at room temperature for at least 30 minutes (minimum lysis times and volumes may vary between cell types). Samples may be stored at room temperature for several hours or at < -20°C for longer-term storage, if desired.
3. Measure flash luminescence using a luminometer set to inject 60 µl of 1X Assay Buffer with Substrate per well (ideally followed by a 1 second shaking). A 1-second integration time is usually sufficient.

If an injecting luminometer is not available, manually add 60 µl per well of 1X Assay Buffer with Substrate using a multi-channel pipettor, and then read in a luminometer after a set delay period of at least 2 minutes and more preferably 5-10 minutes.

Note: The "flash" signal is exceptionally strong and decays rapidly during the first 1-2 minutes before entering a "glow" phase. For maximum signal stability in glow reactions (*e.g.* when reading an entire plate at once without an injecting luminometer), set a delay of 10 minutes between addition of Substrate and reading of the samples. However, shorter delays provide higher signal strength (if required) and adequate signal stability for most applications. See Appendix, Section: A1 for additional information on RapidReporter Kinetics.

References

1. Voon D.C., *et al.,* 2005. *Nucleic Acids Res.* 33(3): e27.

Patent & Licensing Information

RapidReporter Vectors and RapidReporter Assay Kits are developed, designed and sold for research purposes only. All due care and attention should be exercised in the handling of the materials described in these kits.

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Gaussia Luciferase Assay Kits: Patent pending. Sold under license from GeneStream Pty Ltd.

Appendix

Section A. Troubleshooting Guide

PROBLEM/ QUESTION	POSSIBLE CAUSE	RECOMMENDATION
High background luminescence	Light leakage from other wells	Use white-walled plates. Set up your assay so that your luminometer measures the stimulated samples AFTER the corresponding controls (as the brightness of sample wells can affect the readings of subsequent wells, especially if the next well is an unstimulated sample or a blank). Alternately, leave spaces between wells to avoid light leakage.
No signal or weak signal (including for positive control)	Failed transfection	Optimize transfection protocol and use high-quality, endotoxin-free DNA. Thaw and use fresh cells. Expression can be improved by optimizing the cell's health. Increase seeding density or time of culture to allow cells to proliferate and adhere more tightly.
	Incomplete cell lysis	Ensure cell lysis lasts for at least 30 minutes. Try longer lysis or a freeze-thaw cycle if cells are resistant to lysis.
No signal or weak signal in samples (but the positive control signal is OK)	Sequence error	Check the sequence around your cloned element (for MCS vectors only).
	Low expression in cells of interest	Thaw fresh cells or try a different cell type. Prepare fresh stimulant. Use flash reactions rather than glow.
	Luminometer	Use flash reactions whenever possible. Otherwise, reduce the time lag for manual injections and measure rows or columns individually. Increase the gain of the luminometer.
No induction/repression	Stimulator/inhibitor not effective	Prepare fresh stock. Thaw fresh cells. Try another cell type.
High variance within replicates	Light leakage	See above.
	Variations in time delay	Use flash reactions with an injecting luminometer or prolong the time delay before reading samples so that you enter the stable glow phase, see Figure 4, page 20.
Cells detach from assay well surface		Ensure gentle removal of media from assay wells just prior to addition of 1X Lysis Buffer.

PROBLEM/ QUESTION	POSSIBLE CAUSE	RECOMMENDATION
Can the system be used *in vivo*?		Because RapidReporter requires a pH that is higher than required *in vivo*, it is not suitable for this type of application.
How do I normalize the reaction to account for differences in transfection efficiency?		Rather than attempting to control for variability we believe it is best to eliminate the possibility of variability in transfection efficiency. Following our protocol, a single flask of cells is transfected and this flask is split into the wells of a multi-well plate., such that all wells contain the same number of cells from the same transfection. Thus, the reporter activity of treated cells is expressed relative to that of the untreated cells.

Section A1: RapidReporter Signal Decay

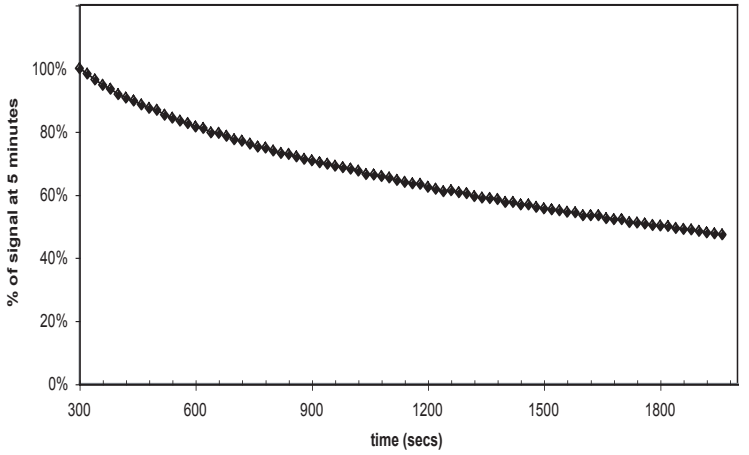

Figure 4: Signal Decay of RapidReporter *Gaussia* luciferase after 5 minutes
The RapidReporter Assay measurements were taken 5 minutes after reagent addition. We recommend using a flash reaction with an injecting luminometer. If you are using a manual injector, we recommend reading the results after 10 minutes so that you are reading in the glow phase. The *Gaussia* luciferase utilized with RapidReporter has a half life of 30 minutes.

Section B. Related Products

TransAM™ Kits	Unit	Catalog No.
TransAM™ NFκB p50	1 x 96 rxns	41096
	5 x 96 rxns	41596
TransAM™ NFκB p65	1 x 96 rxns	40096
	5 x 96 rxns	40596
TransAM™ NFκB Family	2 x 96 rxns	43296
TransAM™ CREB	1 x 96 rxns	42096
	5 x 96 rxns	42596
TransAM™ CREB	1 x 96 rxns	42096
	5 x 96 rxns	42596
TransAM™ pCREB	1 x 96 rxns	43096
	5 x 96 rxns	43596
TransAM™ c-Myc	1 x 96 rxns	43396
	5 x 96 rxns	43896
TransAM™ Elk-1	1 x 96 rxns	44396
	5 x 96 rxns	44896
TransAM™ MAPK Family	2 x 96 rxns	47296
TransAM™ STAT Family	2 x 96 rxns	42296
TransAM™ STAT3	1 x 96 rxns	45196
	5 x 96 rxns	45696

Sample Preparation	Format	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™	25 rxns	53001
ChIP-IT™ without controls	25 rxns	53004
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Enzymatic without controls	25 rxns	53007
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Shearing Kit for sonication	10 rxns	53002
Enzymatic Shearing Kit	10 rxns	53005
ChIP-IT™ Control Kit - Human	5 rxns	53010
Ready-to-ChIP HeLa Chromatin	10 rxns	53015

DNA Methylation	Format	Catalog No.
MethylDetector™	50 rxns	55001

SUMOylation	Format	Catalog No
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

Gene Silencing	Format	Catalog No.
Custom gripNA™ Probe	200 nmol	24001

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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