TECHNICAL MANUAL

pGL4 Luciferase Reporter Vectors

Instructions for Use of Products
E1310, E1320, E1340, E1350, E1360, E6651, E6661, E6671, E6681, E6691, E6701, E6711, E6721, E6731, E6741, E6751, E6761, E6771, E6881, E6891, E6901, E6911, E6921, E6931, E6941, E6951, E6961, E6971, E6981, E6991, E7501, E7511, E7521, E8411, E8421, E8431, E8441, E8451, E8461, E8471, E8481 and E8491

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Promega

pGL4 Luciferase Reporter Vectors

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Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The pGL4 Luciferase Reporter Vectors[a–e] are the next generation of reporter gene vectors optimized for expression in mammalian cells. Numerous configurations of pGL4 Vectors are available, including those with the synthetic firefly *luc2* (*Photinus pyralis*) and *Renilla hRluc* (*Renilla reniformis*) genes, which have been codon optimized for more efficient expression in mammalian cells. Furthermore, both the reporter genes and the vector backbone, including the *bla* (β-lactamase or Amp^r) and mammalian selectable marker genes for hygromycin (Hygro or Hyg^r), neomycin (Neo or Neo^r) and puromycin (Puro or Puro^r), have been engineered to reduce the number of consensus transcription factor binding sites, reducing background and the risk of anomalous transcription.

The pGL4 Vector backbone, provided with a choice of *luc2* or *hRluc* genes, is also supplied with two Rapid Response™ Luciferase Reporter genes for each luciferase gene. The proteins encoded by these Rapid Response™ Luciferase genes respond more quickly and with greater magnitude to changes in transcriptional activity than their more stable counterparts.

1. Description (continued)

The pGL4 Vector family includes:

- Basic vectors with no promoter that contain a multiple cloning region for cloning a promoter of choice
- Vectors containing a minimal promoter
- Vectors containing response elements and a minimal promoter
- Promoter-containing vectors that can be used as expression controls or as co-reporter vectors

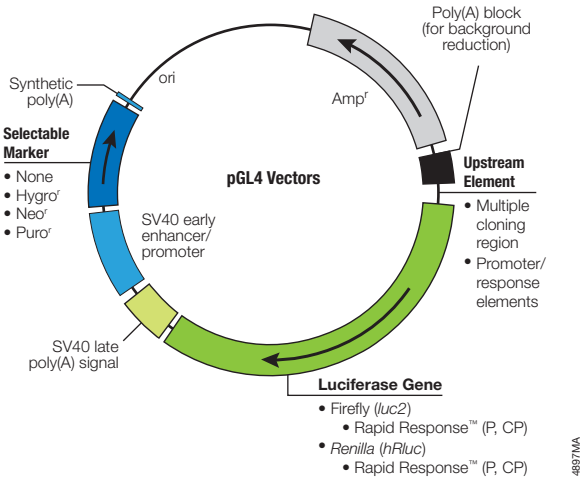


Figure 1. Generic pGL4 Vector map showing the variety of features.

Advantages of the pGL4 Vectors include:

Improved sensitivity and biological relevance due to:

- **Increased reporter gene expression:** Codon optimization of synthetic genes for mammalian expression
- **Reduced background and risk of expression artifacts:** Removal of cryptic DNA regulatory elements and transcription factor binding sites
- **Improved temporal response:** Rapid Response™ technology available using destabilized luciferase genes

Enhanced usability and convenience:

- **Flexible detection options:** Choice of either synthetic *luc2* (*Photinus pyralis*) or *hRluc* (*Renilla reniformis*) reporter genes
- **Easy transition from transient to stable cells:** Choice of mammalian selectable markers
- **Easy transfer from one vector to another:** Common multiple cloning site and a unique SfiI transfer scheme

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
pGL4.10[*luc2*] Vector[a–c]	20µg	E6651
pGL4.11[*luc2P*] Vector[a–c]	20µg	E6661
pGL4.12[*luc2CP*] Vector[a–c]	20µg	E6671
pGL4.13[*luc2*/SV40] Vector[a–c]	20µg	E6681
pGL4.14[*luc2*/Hygro] Vector[a–d]	20µg	E6691
pGL4.15[*luc2P*/Hygro] Vector[a–d]	20µg	E6701
pGL4.16[*luc2CP*/Hygro] Vector[a–d]	20µg	E6711
pGL4.17[*luc2*/Neo] Vector[a–d]	20µg	E6721
pGL4.18[*luc2P*/Neo] Vector[a–d]	20µg	E6731
pGL4.19[*luc2CP*/Neo] Vector[a–d]	20µg	E6741
pGL4.20[*luc2*/Puro] Vector[a–d]	20µg	E6751
pGL4.21[*luc2P*/Puro] Vector[a–d]	20µg	E6761
pGL4.22[*luc2CP*/Puro] Vector[a–d]	20µg	E6771
pGL4.23[*luc2*/minP] Vector[a–d]	20µg	E8411
pGL4.24[*luc2P*/minP] Vector[a–c]	20µg	E8421
pGL4.25[*luc2CP*/minP] Vector[a–c]	20µg	E8431
pGL4.26[*luc2*/minP/Hygro] Vector[a–c]	20µg	E8441
pGL4.27[*luc2P*/minP/Hygro] Vector[a–d]	20µg	E8451
pGL4.28[*luc2CP*/minP/Hygro] Vector[a–d]	20µg	E8461
pGL4.29[*luc2P*/CRE/Hygro] Vector[a–d]	20µg	E8471
pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector[a–d]	20µg	E8481
pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector[a–d]	20µg	E8491
pGL4.33[*luc2P*/SRE/Hygro] Vector[a–d]	20µg	E1340
pGL4.34[*luc2P*/SRF-RE/Hygro] Vector[a–d]	20µg	E1350
pGL4.36[*luc2P*/MMTV/Hygro] Vector[a–d]	20µg	E1360
pGL4.50[*luc2*/CMV/Hygro] Vector[a–d]	20µg	E1310
pGL4.51[*luc2*/CMV/Neo] Vector[a–d]	20µg	E1320
pGL4.70[*hRluc*] Vector[a,e]	20µg	E6881
pGL4.71[*hRlucP*] Vector[a,e]	20µg	E6891
pGL4.72[*hRlucCP*] Vector[a,e]	20µg	E6901
pGL4.73[*hRluc*/SV40] Vector[a,e]	20µg	E6911
pGL4.74[*hRluc*/TK] Vector[a,e]	20µg	E6921
pGL4.75[*hRluc*/CMV] Vector[a,e]	20µg	E6931
pGL4.76[*hRluc*/Hygro] Vector[a,d,e]	20µg	E6941
pGL4.77[*hRlucP*/Hygro] Vector[a,d,e]	20µg	E6951
pGL4.78[*hRlucCP*/Hygro] Vector[a,d,e]	20µg	E6961
pGL4.79[*hRluc*/Neo] Vector[a,d,e]	20µg	E6971

2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
pGL4.80[*hRlucP*/Neo] Vector[a,d,e]	20µg	E6981
pGL4.81[*hRlucCP*/Neo] Vector[a,d,e]	20µg	E6991
pGL4.82[*hRluc*/Puro] Vector[a,d,e]	20µg	E7501
pGL4.83[*hRlucP*/Puro] Vector[a,d,e]	20µg	E7511
pGL4.84[*hRlucCP*/Puro] Vector[a,d,e]	20µg	E7521

Available Separately

PRODUCT	SIZE	CAT.#
pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector[a–d]	20µg	C9351

The *CheckMate™/Flexi® Vector Mammalian Two-Hybrid System Technical Manual*, #TM283, available at: **www.promega.com/tbs**, contains protocol information for use of the pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector.

Storage Conditions: Store the pGL4 Luciferase Reporter Vectors at –20°C.

3. General Considerations

3.A. The pGL4 Vector Backbone

To reduce the risk of anomalous expression and increase the reliability of reporter gene expression, the pGL3 Vector region upstream of the reporter gene was re-engineered to create the pGL4 Vectors. The pGL3 Vector region from the start of the reporter gene (the NcoI restriction site) to the bacterial origin of replication sequence was also redesigned. The modifications to this region include: a greatly reduced number of consensus transcription factor binding sites (Figure 2); a redesign of the multiple cloning region; removal of the f1 origin of replication; deletion of an intronic sequence; and a reduction in the number of promoter modules. (Promoter modules are composite regulatory elements consisting of at least two transcription factor binding sites separated by a spacer. Promoter modules can have synergistic or antagonistic functions (1).)

Note: The synthetic Amp^r in the pGL4 Vectors expresses a protein sequence identical to that expressed by the Amp^r gene in the pGL3 Vectors.

The only regions of the pGL4 Vector backbone not optimized for reduced anomalous expression are the SV40 late poly(A) signal downstream of the reporter gene and the bacterial origin of replication.

Modifications to the pGL4 Vectors have resulted in an improved signal-to-background ratio. Some of these improved ratios are presented in Table 1, where several pGL4 Vectors are compared to their controls (the corresponding promoterless vectors), and the fold increase in the signal-to-background ratio is compared for the pGL4 Vectors and previous generations of reporter vectors (e.g., pGL3 and phRL Vectors).

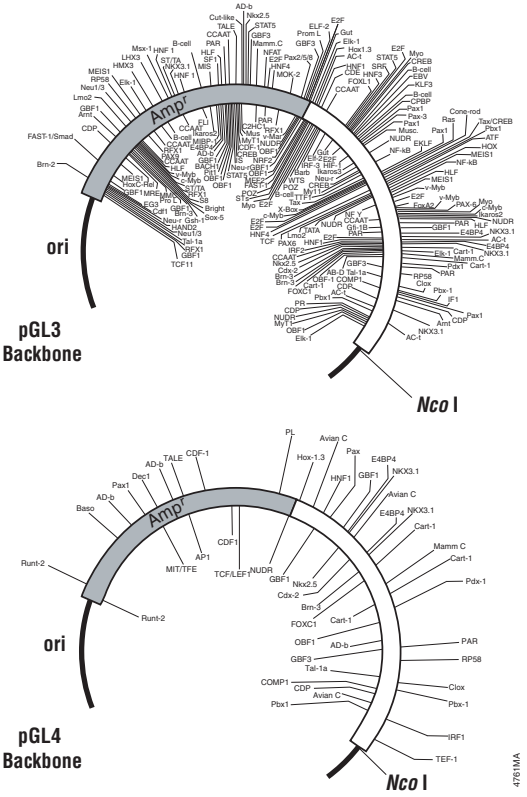


Figure 2. Reduced number of consensus transcription factor binding sites for the pGL4 Vectors. The number of consensus transcription factor binding sites identified in the pGL3 Vector backbone has been greatly reduced in the pGL4 Vector backbone.

Note: The SV40, HSV-TK and CMV promoters contain several consensus transcription factor binding sites. Due to the complex nature of the promoters these sites were not altered. However, the number and type of consensus transcription factor binding sites vary depending on the promoter. For maximum reduction of anomalous expression in your assay system, we recommend that you evaluate the promoters to determine the one that results in the lowest level of anomalous expression with your system.

Synthetic Poly(A) Signal/Transcriptional Pause Site for Background Reduction

All pGL4 Vectors contain a synthetic poly(A) signal/transcriptional pause site (2) located upstream of either the multiple cloning region (in promoterless vectors) or the SV40, CMV or HSV-TK promoter (in promoter-containing vectors). The synthetic poly(A) signal/transcriptional pause site is present to reduce the effects of spurious transcription on luciferase reporter gene expression.

3.A. The pGL4 Vector Backbone (continued)

Table 1. Signal-to-Background Comparison for Several of the pGL4, pGL3 and phRL Vectors.[1]

Vectors	Signal-to-Background Ratio[1]	Fold Increase for pGL4 Vectors[2]
pGL4.13*[luc2*/SV40] vs. pGL4.10[*luc2*]	3,200 ± 340	3.7
pGL3-Control vs. pGL3-Basic	670± 57	
pGL4.73[*hRluc*/SV40] vs. pGL4.70[*hRluc*]	630 ± 78	32
phRL-SV40 vs. phRL-null	19 ± 2	
pGL4.74[*hRluc*/TK] vs. pGL4.70[*hRluc*]	79 ± 8	33
phRL-TK vs. phRL-null	2.3 ± 0.9	
pGL4.75[*hRluc*/CMV] vs. pGL4.70[*hRluc*]	1,100 ± 120	64
phRL-CMV vs. phRL-null	17 ± 2	

[1]To generate signal-to-background ratios the luciferase-containing vectors were transfected into CHO cells. At 24 hours post-transfection the cells were lysed, luminescent signals were generated using the Dual-Luciferase® Assay System and the relative light units were corrected for transfection efficiency, yielding "normalized signals". Signal-to-background ratio = signal from promoter-containing vector/signal from corresponding promoterless vector.

[2]Fold increase for pGL4 Vectors = (signal-to-background ratio from pGL4 Vector/signal-to-background ratio from corresponding pGL3 or phRL Vector) − 1. The experiment was repeated in CHO and other cell lines (HeLa, NIH/3T3 and HEK 293 cells) generating similar results.

3.B. The pGL4 Reporter Genes

To increase expression and reliability of the firefly luciferase reporter, a synthetic firefly luciferase gene, *luc2*, has been engineered. The gene was synthetically redesigned by changing the codons to those most frequently used in mammalian cells while simultaneously removing most of the consensus sequences for transcription factor binding sites (Figure 3). Additionally, the number of predicted promoter modules present within the *luc+* reporter gene (and thought to cause anomalous expression) has been reduced to a single module in the *luc2* gene. To maintain the integrity of the firefly luciferase protein sequence from the *luc+* gene to the *luc2* gene, some of the consensus transcription factor binding sites and promoter modules were not removed. In the transfection experiment shown in Figure 4, the synthetic firefly *luc2* luciferase gene displayed an increase in expression compared to *luc+*. To ensure that the synthetic construction affected only expression, both the *luc+* and *luc2* genes were cloned into the pGL3-Control Vector. The vectors were co-transfected with an internal control for assessing transfection efficiency, and after 24 hours the luminescence (in relative light units) was measured and normalized to the control. When compared to *luc+*, the *luc2* gene demonstrated a 4.1- to 11.8-fold increase in expression for the four mammalian cell lines tested.

For more information on the *hRluc* gene, please refer to the *Synthetic* Renilla *Luciferase Reporter Vectors Technical Manual,* #TM237. See Table 2 for a list of the features in each pGL4 Vector.

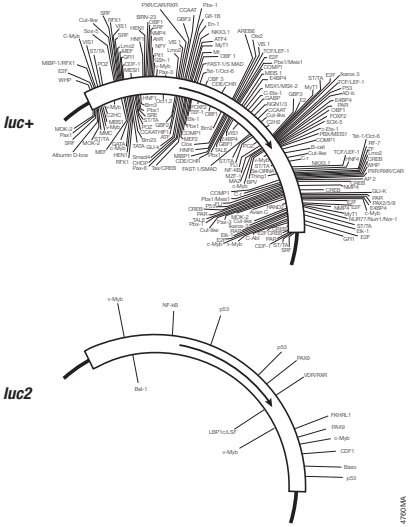


Figure 3. Reduced number of consensus transcription factor binding sites for the *luc2* gene. The number of consensus transcription factor binding sites identified in the *luc+* gene (top) have been greatly reduced in the *luc2* gene (below), while conserving the protein sequence.

3.C. Distinguishing Features of the pGL4 Vectors

The following features are available with the pGL4 Vectors.

Reporter Genes:

- Firefly luciferase (*luc2*) or *Renilla* luciferase (*hRluc*)
- Regular or Rapid Response™ genes

Upstream Elements:

- Promoterless basic vectors
- Vectors with minimal promoters
- Vectors containing response elements and minimal promoters
- Vectors with constitutive promoters such as SV40, HSV-TK and CMV

Selectable Markers:

- Hygro
- Neo
- Puro

3.C. Distinguishing Features of the pGL4 Vectors (continued)

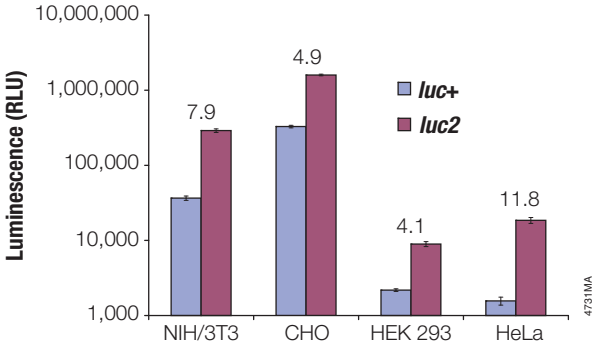


Figure 4. The firefly *luc2* gene displays higher expression than *luc*+. The *luc2* gene was cloned into the pGL3-Control Vector (Cat.# E1741), replacing the *luc*+ gene. Thus both firefly luciferase genes were in the same pGL3-Control Vector backbone. The two vectors containing either of the firefly luciferase genes were co-transfected into NIH/3T3, CHO, HEK 293 and HeLa cells using the phRL-TK Vector (Cat.# E6241) for a transfection control. Twenty-four hours post-transfection the cells were lysed with Passive Lysis Buffer (Cat.# E1941) and luminescence was measured using the Dual-Luciferase® Assay System (Cat.# E1910). Luminescence (relative light units) was normalized to the *Renilla* luciferase expression from the phRL-TK Vector transfection control. The fold increase in expression values is listed above each pair of bars. A repeat of this experiment yielded similar results.

The Rapid Response™ pGL4 Vectors

Computational models predict that genetic reporters with reduced intracellular stability will yield faster response to changes in transcriptional rate and an increase in the relative magnitude of the response (3). Destabilized reporter proteins (i.e., those with faster protein degradation rates) are therefore expected to be more responsive and better suited to monitor rapid processes (such as promoter activation and repression) than those with slower degradation rates.

To generate reporter proteins that have increased protein degradation rates (i.e., destabilized reporters) one or both of two different protein degradation sequences have been incorporated into the synthetic firefly *luc2* and *Renilla hRluc* luciferase genes. The first degradation sequence, PEST, is a forty-amino acid sequence isolated from the C-terminal region of mouse ornithine decarboxylase (4). The second degradation sequence includes CL1 and PEST. CL1, originally isolated from yeast, has also been shown to increase protein degradation (5). The codons within the PEST and CL1-PEST sequences have been optimized for expression in mammalian cells, and the number of consensus transcription factor binding sites has been reduced. To reflect the changes in these two protein degradation sequences they have been designated hPEST and hCL1-hPEST. The Rapid Response™ pGL4 Reporter gene design is shown in Figure 5.

As the result of inclusion of the protein degradation sequences in the Rapid Response™ pGL4 Vectors, the rate of reporter response to cellular stimuli has increased. In Figure 6 and Table 3 increased rates of reporter response in the CRE (cAMP response element) model system are demonstrated. For the firefly luciferase-based Rapid Response™ pGL4 Reporter Vectors (pGL4.11[*luc2P*] and (pGL4.12[*luc2CP*]), the rate of reporter response for the first two hours

Table 2. Features of the pGL4 Vectors.

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter/ Response Element	Mammalian Selectable Marker
pGL4.10[*luc2*]	Yes	*luc2*[1]	No	No	No
pGL4.11[*luc2P*]	Yes	"	hPEST	No	No
pGL4.12[*luc2CP*]	Yes	"	hCL1-hPEST	No	No
pGL4.13[*luc2*/SV40]	No	"	No	SV40	No
pGL4.14[*luc2*/Hygro]	Yes	"	No	No	Hygro
pGL4.15[*luc2P*/Hygro]	Yes	"	hPEST	No	Hygro
pGL4.16[*luc2CP*/Hygro]	Yes	"	hCL1-hPEST	No	Hygro
pGL4.17[*luc2*/Neo]	Yes	"	No	No	Neo
pGL4.18[*luc2P*/Neo]	Yes	"	hPEST	No	Neo
pGL4.19[*luc2CP*/Neo]	Yes	"	hCL1-hPEST	No	Neo
pGL4.20[*luc2*/Puro]	Yes	"	No	No	Puro
pGL4.21[*luc2P*/Puro]	Yes	"	hPEST	No	Puro
pGL4.22[*luc2CP*/Puro]	Yes	"	hCL1-hPEST	No	Puro
pGL4.23[*luc2*/minP]	Yes	"	No	minP	No
pGL4.24[*luc2P*/minP]	Yes	"	hPEST	"	No
pGL4.25[*luc2CP*/minP]	Yes	"	hCL1-hPEST	"	No
pGL4.26[*luc2*/minP/Hygro]	Yes	"	No	"	Hygro
pGL4.27[*luc2P*/minP/Hygro]	Yes	"	hPEST	"	Hygro
pGL4.28[*luc2CP*/minP/Hygro]	Yes	"	hCL1-hPEST	"	Hygro
pGL4.29[*luc2P*/CRE/Hygro]	No	"	hPEST	CRE	Hygro
pGL4.30[*luc2P*/NFAT-RE/Hygro]	No	"	hPEST	NFAT-RE	Hygro
pGL4.31[*luc2P*/*GAL4*UAS/Hygro]	No	"	hPEST	*GAL4*UAS	Hygro
pGL4.32[*luc2P*/NF-κB-RE/Hygro]	No	"	hPEST	NF-κB-RE	Hygro
pGL4.33[*luc2P*/SRE/Hygro]	No	"	hPEST	SRE	Hygro
pGL4.34[*luc2P*/SRF-RE/Hygro]	No	"	hPEST	SRF-RE	Hygro
pGL4.36[*luc2P*/MMTV/Hygro]	No	"	hPEST	MMTV	Hygro
pGL4.50[*luc2*/CMV/Hygro]	No	"	No	CMV	Hygro
pGL4.51[*luc2*/CMV/Neo]	No	"	No	CMV	Neo
pGL4.70[*hRluc*]	Yes	*hRluc*[2]	No	No	No
pGL4.71[*hRlucP*]	Yes	"	hPEST	No	No
pGL4.72[*hRlucCP*]	Yes	"	hCL1-hPEST	No	No
pGL4.73[*hRluc*/SV40]	No	"	No	SV40	No
pGL4.74[*hRluc*/TK]	No	"	No	HSV-TK	No
pGL4.75[*hRluc*/CMV]	No	"	No	CMV	No
pGL4.76[*hRluc*/Hygro]	Yes	"	No	No	Hygro
pGL4.77[*hRlucP*/Hygro]	Yes	"	hPEST	No	Hygro
pGL4.78[*hRlucCP*/Hygro]	Yes	"	hCL1-hPEST	No	Hygro
pGL4.79[*hRluc*/Neo]	Yes	"	No	No	Neo
pGL4.80[*hRlucP*/Neo]	Yes	"	hPEST	No	Neo
pGL4.81[*hRlucCP*/Neo]	Yes	"	hCL1-hPEST	No	Neo
pGL4.82[*hRluc*/Puro]	Yes	"	No	No	Puro
pGL4.83[*hRlucP*/Puro]	Yes	"	hPEST	No	Puro
pGL4.84[*hRlucCP*/Puro]	Yes	"	hCL1-hPEST	No	Puro

[1]*luc2* is the synthetic firefly luciferase gene.

[2]*hRluc* is the synthetic *Renilla* gene.

3.C. Distinguishing Features of the pGL4 Vectors (continued)

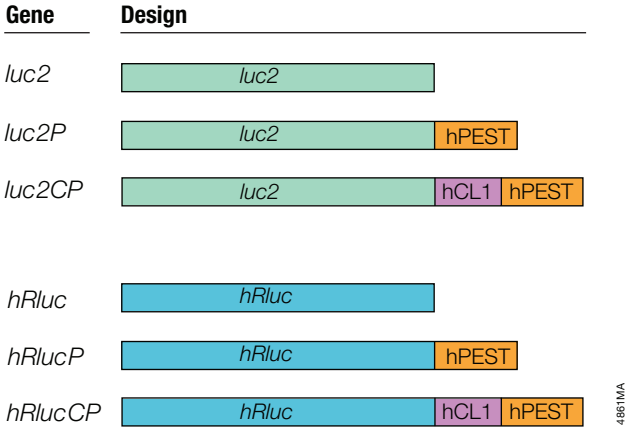


Figure 5. Gene design of the Rapid Response™ pGL4 Reporter Vectors.

post-induction increased one-and twofold, respectively, over the *luc2* control (pGL4.10[*luc2*]). Similar results for the same time frame were detected for the *Renilla*-based Rapid Response™ pGL4 Reporter Vectors (pGL4.71[*hRlucP*] and pGL4.72[*hRlucCP*]). The rate of response for the *hRlucP*- and *hRlucCP*-destabilized *Renilla* reporters increased one- and threefold, respectively, when compared to the nondestabilized *hRluc* reporter.

A consequence of inclusion of the degradation sequences is that the destabilized luciferase proteins do not accumulate in the cell to the same extent as the nondestabilized luciferase-containing controls. As a result, destabilized reporter proteins typically generate lower signal intensities (Figure 7). The *luc2P* and *luc2CP* proteins displayed 18.7% and 3.9%, respectively, of the relative light units obtained using the firefly luciferase gene *luc2*. Similar reductions in relative light intensities were displayed by the *Renilla* luciferase-based Rapid Response™ pGL4 Reporter Vectors. The *Renilla* reporter proteins *hRlucP* and *hRlucCP* displayed 40% and 5.8%, respectively, of the relative light units of the *hRluc* control. Luminescence obtained using the Rapid Response™ pGL4 Reporter Vectors can vary depending on the mammalian cell line and experimental conditions used, thus optimization is recommended.

Table 3. Increase in Reporter Response for the Rapid Response™ pGL4 Reporter Vectors (results calculated from the data shown in Figure 6)**.**

Vector	Induction at 2 Hours[1]	Fold Increase in Reporter Response Rate[2]
pGL4.10[*luc2*]	6.6	–
pGL4.11[*luc2P*]	13.8	1
pGL4.12[*luc2CP*]	21.6	2
pGL4.70[*hRluc*]	2.7	–
pGL4.71[*hRlucP*]	6.3	1
pGL4.72[*hRlucCP*]	12.2	3

[1]Induction = signal from induced samples/signal from noninduced samples.
[2]Reporter Response Rate = (induction of Rapid Response™ Reporter/induction of a regular reporter) – 1.

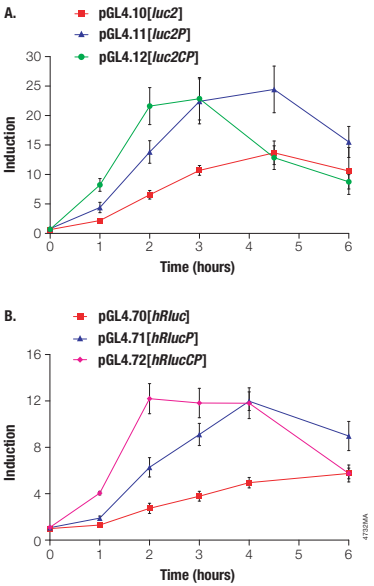


Figure 6. Increase in reporter response rate. To determine the increase in reporter response rate for the Rapid Response™ pGL4 Reporter Vectors, a DNA segment containing multiple CREs (cAMP response element) and a minimal HSV-TK promoter were cloned into each of the four Rapid Response™ pGL4 Reporter Vectors (pGL4.11[*luc2P*], pGL4.12[*luc2CP*], pGL4.71[*hRlucP*] and pGL4.72[*hRlucCP*]) and the two control vectors (pGL4.10[*luc2*] and pGL4.70[*hRluc*]). The CRE-containing vectors were then transiently transfected into HEK 293 cells. Twenty-four hours later 100µM of RO (RO-20-1724) and 1µM of ISO (isoproterenol hydrochloride) were added to the transiently transfected cells to induce reporter gene expression. RO alone (100µM) was added to a subset of the wells to serve as a noninduced control. Cells were harvested, lysed and assayed with either the Luciferase Assay System (Cat.# E1500; Panel A) or the *Renilla* Luciferase Assay System (Cat.# E2810; Panel B). Induction was calculated by dividing the relative light units obtained from the induced wells by the relative light units obtained from noninduced wells. Two repeats of this experiment yielded similar results.

The Promoterless pGL4 Vectors

The promoterless pGL4 Vectors contain no enhancer or promoter elements. These vectors, which contain a multiple cloning region immediately upstream of the luciferase reporter gene, can be used to clone in a desired regulatory element(s) to drive expression of the reporter gene.

The Minimal Promoter pGL4 Vectors

The minimal promoter (minP) vectors contain a TATA-box promoter element immediately upstream of the luciferase reporter gene and immediately downstream of the multiple cloning region. These vectors can be used to clone in a desired promoterless-response element to drive expression of the reporter gene. The minimal TATA promoter has low basal activity and allows for sensitive response element activity measurements.

3.C. Distinguishing Features of the pGL4 Vectors (continued)

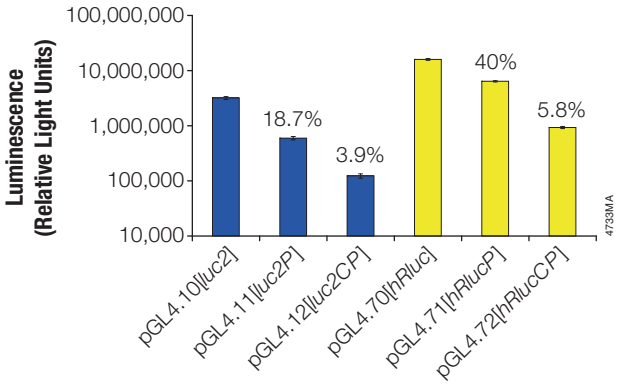


Figure 7. Relative expression of Rapid Response™ pGL4 luciferase genes. To determine the relative expression of the Rapid Response™ pGL4 luciferase genes, a DNA segment containing multiple CREs (cAMP response element) and a minimal HSV-TK promoter was cloned into each of the four Rapid Response™ pGL4 Reporter Vectors (pGL4.11[*luc2P*], pGL4.12[*luc2CP*], pGL4.71[*hRlucP*], and pGL4.72[*hRlucCP*]) and the two control vectors (pGL4.10[*luc2*] and pGL4.70[*hRluc*]), which do not contain degradation sequences. The resulting vectors were co-transfected with a second reporter (transfection control) into HEK 293 cells. The transfection controls for the firefly *luc2*-containing vectors were the phRL-TK Vector and, for the *Renilla hRluc* vectors, the pGL3-Control Vector. Twenty-four hours later the cells were lysed with Passive Lysis Buffer and luminescence (relative light units) was measured using the Dual-Luciferase® Assay System. (The firefly *luc2* data is shown by three bars on the left, while the *hRluc* data is contained in the three bars on the right.) Luminescence (relative light units) was normalized to the transfection control. The effects of the protein degradation sequences on the accumulation of luciferase enzyme are shown as percent expression of the control.

Reporter Vectors for the Study of Cell Signaling

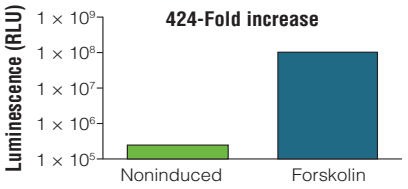
Promega has developed a series of vectors containing response elements or other promoter elements that can be used to study cellular signaling and other events. These vectors can be used in transient transfections or to generate a stable cell line. All vectors use a destabilized *luc2P* firefly luciferase gene, resulting in low backgrounds and high levels of induction. Stable cell selection is possible using hygromycin resistance. Figures 8 and 9 show results from transient transfections using vectors containing response elements. Table 4 provides details on the response element-containing reporter vectors.

Table 4. Details of the Response Element-Containing pGL4 Vectors.

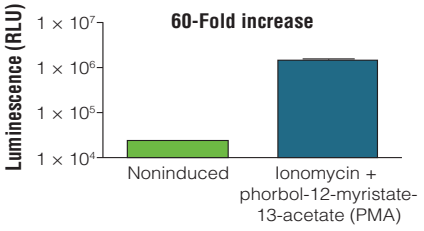
Vector	Sequence of Binding Site Repeat	Signaling Pathway	Selectable Marker
pGL4.29[*luc2P*/CRE/Hygro]	$(TGACGTC)_3$	cAMP/PKA	Hygromycin resistance
pGL4.30[*luc2P*/NFAT-RE/Hygro]	$(GGAGGAAAAACTGTTTCATACAGAAGGCGT)_3$	Calcium/Calcineurin	Hygromycin resistance
pGL4.31[*luc2P*/*GAL4*UAS/Hygro]	$(CGGAGTACTGTCCTCCGA)_5$	Varies (requires binding and activation by GAL4-DNA binding domain)	Hygromycin resistance
pGL4.32[*luc2P*/NF-kB/Hygro]	$(GGGAATTTCC)_5$	NF-kB	Hygromycin resistance
pGL4.33[*luc2P*/SRE/Hygro]	$(AGGATGTCCATATTAGG)_5$	MAPK/ERK	Hygromycin resistance
pGL4.34[*luc2P*/SRF-RE/Hygro]	$(CCATATTAGG)_5$	RhoA	Hygromycin resistance
pGL4.36[*luc2P*/MMTV/Hygro]	Murine Mouse Tumor Virus Long Terminal Repeat (6–9)	Several nuclear receptors including androgen receptor and glucocorticoid receptor	Hygromycin resistance

3.C. Distinguishing Features of the pGL4 Vectors (continued)

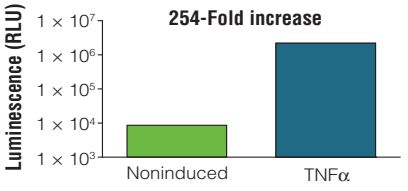
A. pGL4.29[*luc2P*/CRE/Hygro] Vector



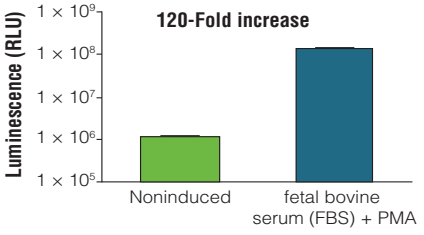
B. pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector



C. pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector



D. pGL4.33[*luc2P*/SRE/Hygro] Vector



E. pGL4.34[*luc2P*/SRF-RE/Hygro] Vector

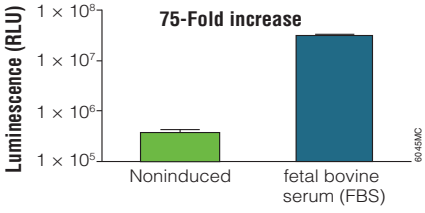


Figure 8. High levels of reporter induction for response element-containing vectors in transient transfections. pGL4.29 (Panel A), pGL4.30 (Panel B), pGL4.32 (Panel C), pGL4.33 (Panel D) and pGL4.34 (Panel E) Vectors were transiently transfected into HEK293 cells. After 4 hours, the pGL4.33 and pGL4.34 Vectors were changed to serum-starved media. After 24 hours, reporter gene expression was induced by adding: 100µM forskolin for 5 hours (Panel A); 1µM ionomycin plus 10ng/ml PMA for 17 hours (Panel B); 20ng/µl of TNFα for 5 hours (Panel C); 20% FBS plus 10ng/ml PMA (Panel D); and 20% FBS for 6 hours (Panel E). Noninduced controls were treated with an equivalent amount of DMSO vehicle in Panels A and B, and with an equivalent amount of standard media for Panels C, D and E. Luciferase activity was measured using the Bright-Glo™ Luciferase Assay System (Cat.# E2610) for Panels A and B, and with the ONE-Glo™ Luciferase Assay System (Cat.# E6110) for Panels C, D and E. The numbers shown above the graph bars represent fold induction for the respective vectors, calculated by dividing the relative light units obtained for the induced wells by the relative light units obtained from noninduced wells (n = 15; standard error bars are shown). Results will vary depending on the cell line and the transfection protocol used.

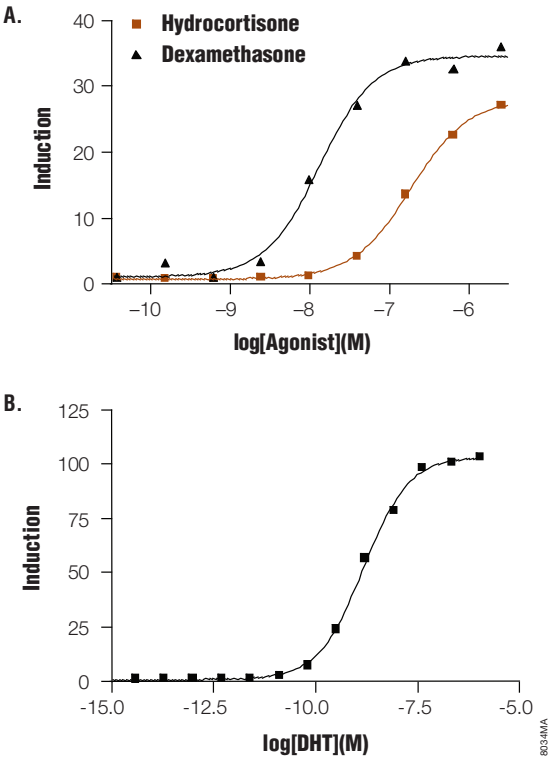


Figure 9. Endogenous receptor activation post-transfection, as measured by luciferase expression. **Panel A.** HeLa cells were transiently transfected with the pGL4.36[*luc2P*/MMTV/Hygro] Vector, then induced with 1:4 serial dilutions of either hydrocortisone or dexamethasone, for 24 hours. **Panel B.** HEK293 cells were transiently transfected with the pGL4.36[*luc2P*/MMTV/Hygro] Vector and a CMV-driven androgen receptor, then induced with 1:5 dilutions of dihydrotestosterone (DHT). For both Panels A and B, luminescence was quantitated with Dual-Glo® Luciferase Assay System (Cat.# E2920) using the GloMax® 96 Microplate Luminometer (Cat.# E6501). Induction was calculated as the average induced divided by the average noninduced firefly luminescence (in RLU or relative light units).

The *GAL4*UAS Reporter Vector

Another response element vector, the pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector is part of the CheckMate™/Flexi® Vector Mammalian Two-Hybrid System. This reporter vector contains five consensus binding sequences, or Upstream Activating Sequences (UAS), for the GAL4 DNA-binding domain (*GAL4*UAS) upstream of a minimal adenoviral promoter and is designed for transcriptional activation of the firefly luciferase by association of the GAL4 DNA-binding and VP16 activation domains bound upstream of the luciferase gene.

3.C. Distinguishing Features of the pGL4 Vectors (continued)

The Internal Control pGL4 Vectors

The pGL4.13[*luc2*/SV40] and pGL4.73[*hRluc*/SV40] Vectors are intended for use as internal control reporters and may be used with most experimental reporter vectors to co-transfect mammalian cells. These vectors contain the SV40 early enhancer/promoter region, which provides strong, constitutive expression of luciferase in a variety of cell types, as well as the SV40 origin of replication, which results in transient, episomal replication in cells expressing the SV40 large T antigen such as COS-1 or COS-7 cells (10).

These promoter-containing vectors may not be appropriate for use in examining the function of a response element of interest. Minimal promoter-containing vectors are better suited for examining a response element of interest.

The pGL4.74[*hRluc*/TK] Vector is intended for use as an internal control reporter and may be used with most experimental reporter vectors to co-transfect mammalian cells. The pGL4.74[*hRluc*/TK] Vector contains the herpes simplex virus thymidine kinase (HSV-TK) promoter upstream of *hRluc* to provide low to moderate levels of *Renilla* luciferase expression in co-transfected mammalian cells of both embryonal and mature mammalian tissues (10,11).

The pGL4.75[*hRluc*/CMV] Vector is intended for use as an internal control reporter and may be used with most experimental reporter vectors to co-transfect mammalian cells. The pGL4.75[*hRluc*/CMV] Vector contains the CMV immediate-early enhancer/promoter region, which provides strong, constitutive expression of *Renilla* luciferase in a variety of cell types. The promiscuous nature of the CMV immediate-early enhancer/promoter has been demonstrated in transgenic mice, where its transcriptional activity was observed in 24 of the 28 murine tissues examined (12).

Selectable Markers

Hygromycin B is an aminoglycosidic antibiotic that inhibits protein synthesis by disrupting translocation and promoting mistranslation at the 80S ribosome. Hygromycin B phosphotransferase (Hygro or Hyg^r) inactivates hygromycin B by phosphorylation. For the selection of stable cell lines, an expression cassette for the gene encoding hygromycin B phosphotransferase is available in the pGL4 Vectors listed as “Hygro” in Table 2, under “Mammalian Selectable Markers”.

G418 is an aminoglycosidic antibiotic that inhibits protein synthesis. Neomycin or aminoglycoside 3´ phosphotransferase (Neo or Neo^r) confers resistance to G418. For the selection of stable cell lines, an expression cassette for the gene encoding neomycin phosphotransferase is available in the pGL4 Vectors listed as “Neo” in Table 2, under “Mammalian Selectable Markers”.

Puromycin dihydrochloride is a nucleoside antibiotic that inhibits protein synthesis. Puromycin-N-acetyltransferase (Puro or Puro^r) confers resistance to puromycin. For the selection of stable cell lines, an expression cassette for the gene encoding puromycin-N-acetyltransferase is available in the pGL4 Vectors listed as “Puro” in Table 2, under “Mammalian Selectable Markers”.

3.D. Common Features of the pGL4 Vectors

SV40 Late Poly(A) Signal

In addition to the features previously listed, each of the pGL4 Vectors contains a SV40 late poly(A) signal downstream of the reporter gene. Polyadenylation signals cause the termination of transcription by RNA polymerase II and signal the addition of approximately 200–250 adenosine residues to the 3´-end of the RNA transcript (13). Polyadenylation has been shown to enhance RNA stability and translation efficiency (14,15). The SV40 late poly(A) signal is extremely efficient and has been shown to increase the steady-state level of RNA approximately fivefold more than the SV40 early poly(A) signal (16,17). The SV40 late poly(A) signal has been positioned 3´ to the reporter gene in the pGL4 Vectors to increase the level of luciferase expression.

Redesigned Multiple Cloning Region

The multiple cloning region of the pGL4 Vectors (Figure 9) has been synthetically constructed and is based on the multiple cloning region of the pGL3 Vectors. However, differences between the two multiple cloning regions exist. The pGL4 Vector multiple cloning region includes the following restriction sites: BglI, SfiI, Acc65I, KpnI, EcoICRI, SacI, NheI, XhoI, EcoRV, BglII, and HindIII. The BglI, SfiI and EcoRV restriction sites have been added to the pGL4 Vector multiple cloning region and are not found in the pGL3 Vector multiple cloning region. The MluI and SmaI restriction sites found in the pGL3 multiple cloning region have not been included in the pGL4 multiple cloning region. The purpose of two BglI/SfiI restriction sites is to be able to move DNA of interest (i.e., response elements, enhancers, promoters, etc.) among the pGL4 Vectors. Additionally, due to the unique DNA recognition properties of BglI and SfiI, the two restriction sites in the pGL4 Vectors offer directionality. Thus transfer between pGL4 Vectors by using either the BglI or SfiI restriction enzymes retains the desired directionality of your DNA fragment of interest.

Note: Additional BglI and EcoRV restriction sites are present in the *hRluc* gene. Therefore, these two enzymes should not be used for cloning into *Renilla*-based pGL4 Vectors.

3.D. Common Features of the pGL4 Vectors (continued)

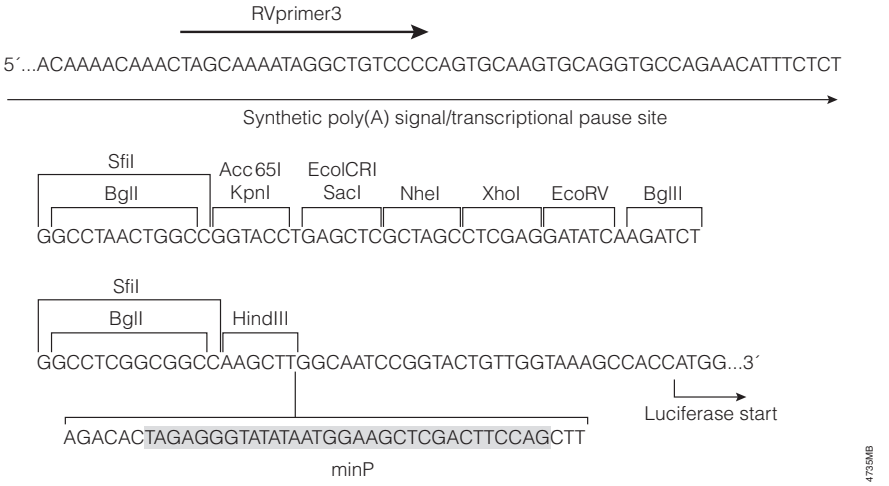


Figure 10. The multiple cloning region of the pGL4 Vectors. This map represents the promoterless pGL4 Vectors but also demonstrates the position and sequence of the minimal promoter, minP, found in the pGL4.23–pGL4.28 Vectors. The minP sequence is shaded in gray. **Note:** The BglI and EcoRV sites are also present in the *hRluc* gene and should not be used for cloning into the *hRluc*- (*Renilla* luciferase-) containing pGL4 Vectors.

4. pGL4 Vector GenBank® Accession Numbers, Sequences, Restriction Enzyme Tables and Maps

Sequence information, vector maps and restriction enzyme tables for the pGL4 Vectors are available online at: **www.promega.com/vectors.** The GenBank®/EMBL accession numbers are listed in Table 5 of this manual.

For each map a selected set of restriction enzyme sites is shown, including those in the multiple cloning regions, EcoRI, which denotes the Rapid Response™ Reporter and BamHI and SalI, which denote the selectable marker regions.

Information for pGL4.31[*luc2P/GAL4*UAS/Hygro] Vector can be found in the *CheckMate™/Flexi® Vector Mammalian Two-Hybrid System Technical Manual*, #TM283 and online at: **www.promega.com/vectors**

Notes:

1. Due to the use of updated software for generating vector maps and restriction enzyme tables, the manner in which we report restriction enzyme cut sites has changed. The location is now reported at the 5´-end of the cut DNA (the base to the right of the cut site). The maps and restriction enzyme tables associated with the pGL4 Vectors have been updated accordingly.
2. The restriction enzyme sites shown on the vector maps were derived from sequence analysis software and have not been verified by restriction enzyme digestion with each enzyme listed. The location given specifies the 5´ end of the cut DNA (the base to the right of the cut site).

Table 5. GenBank®/EMBL Accession Numbers

Vector	GenBank®/EMBL Accession Number
pGL4.10[*luc2*] Vector	AY738222
pGL4.11[*luc2P*] Vector	AY738223
pGL4.12[*luc2CP*] Vector	AY738224
pGL4.13[*luc2*/SV40] Vector	AY738225
pGL4.14[*luc2*/Hygro] Vector	AY864928
pGL4.15[*luc2P*/Hygro] Vector	AY864929
pGL4.16[*luc2CP*/Hygro] Vector	AY864930
pGL4.17[*luc2*/Neo] Vector	DQ188837
pGL4.18[*luc2P*/Neo] Vector	DQ188838
pGL4.19[*luc2CP*/Neo] Vector	DQ188839
pGL4.20[*luc2*/Puro] Vector	DQ188840
pGL4.21[*luc2P*/Puro] Vector	DQ188841
pGL4.22[*luc2CP*/Puro] Vector	DQ188842
pGL4.23[*luc2*/minP] Vector	DQ904455
pGL4.24[*luc2P*/minP] Vector	DQ904456
pGL4.25[*luc2CP*/minP] Vector	DQ904457
pGL4.26[*luc2*/minP/Hygro] Vector	DQ904458
pGL4.27[*luc2P*/minP/Hygro] Vector	DQ904459
pGL4.28[*luc2CP*/minP/Hygro] Vector	DQ904460
pGL4.29[*luc2P*/CRE/Hygro] Vector	DQ904461
pGL4.30[*luc2P*/NFAT-RE/Hygro] Vector	DQ904462
pGL4.31[*luc2P*/*GAL4*UAS/Hygro] Vector	DQ487213
pGL4.32[*luc2P*/NF-κB-RE/Hygro] Vector	EU581860
pGL4.33[*luc2P*/SRE/Hygro] Vector	FJ773212
pGL4.34[*luc2P*/SRF-RE/Hygro] Vector	FJ773213
pGL4.36[*luc2P*/MMTV/Hygro] Vector	FJ773214
pGL4.50[*luc2*/CMV/Hygro] Vector	EU921840
pGL4.51[*luc2*/CMV/Neo] Vector	EU921841
pGL4.70[*hRluc*] Vector	AY738226
pGL4.71[*hRlucP*] Vector	AY738227
pGL4.72[*hRlucCP*] Vector	AY738228
pGL4.73[*hRluc*/SV40] Vector	AY738229
pGL4.74[*hRluc*/TK] Vector	AY738230
pGL4.75[*hRluc*/CM*V*] Vector	AY738231
pGL4.76[*hRluc*/Hygro] Vector	AY864931
pGL4.77[*hRlucP*/Hygro] Vector	AY864932
pGL4.78[*hRlucCP*/Hygro] Vector	AY864933
pGL4.79[*hRluc*/Neo] Vector	DQ188843
pGL4.80[*hRlucP*/Neo] Vector	DQ188844
pGL4.81[*hRlucCP*/Neo] Vector	DQ188845
pGL4.82[*hRluc*/Puro] Vector	DQ188846
pGL4.83[*hRlucP*/Puro] Vector	DQ188847
pGL4.84[*hRlucCP*/Puro] Vector	DQ188848

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6. Related Products

Renilla Luciferase Assay Systems

Product	Size	Cat.#
EnduRen™ Live Cell Substrate	0.34mg	E6481
	3.4mg	E6482
	34mg	E6485
ViviRen™ Live Cell Substrate	0.37mg	E6491
	3.7mg	E6492
	37mg	E6495
Renilla Luciferase Assay System	100 assays	E2810
	1,000 assays	E2820

Luciferase Reporter Cell Lines

Product	Size	Cat.#
GloResponse™ CRE-*luc2P* HEK293 Cell Line	2 vials	E8500
GloResponse™ NFAT-RE-*luc2P* HEK293 Cell Line	2 vials	E8510
GloResponse™ NF-κB-RE-*luc2P* HEK293 Cell Line	2 vials	E8520

Firefly Luciferase Assay Systems

Product	Size	Cat.#
ONE-Glo™ Luciferase Assay System	10ml	E6110
	100ml	E6120
	1L	E6130
Bright-Glo™ Luciferase Assay System	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650
Steady-Glo® Luciferase Assay System	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Luciferase Assay System	100 assays	E1500
Luciferase 1000 Assay System	1,000 assays	E4550
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030

6. Related Products (continued)

Firefly and *Renilla* (Dual-Reporter) Luciferase Assay Systems

Product	Size	Cat.#
Dual-Glo® Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter 1000 Assay System	1,000 assays	E1980

Luminometers

Product	Size	Cat.#
GloMax® 20/20 Luminometer	1 each	E5311
GloMax® 20/20 Luminometer w/Single Auto-Injector	1 each	E5321
GloMax® 20/20 Luminometer w/Dual Auto-Injector	1 each	E5331
GloMax® 96 Microplate Luminometer	1 each	E6501
GloMax® 96 Microplate Luminometer w/Single Injector	1 each	E6511
GloMax® 96 Microplate Luminometer w/Dual Injectors	1 each	E6521

Antibiotic

Product	Size	Cat.#
Antibiotic G-418 Sulfate	5g	V7983

7. Summary of Changes

The following changes were made to the 3/15 revision of this document:

1. Updated patent/license statements and related products.

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[b]U.S. Pat. No. 5,670,356.

[c]U.S. Pat. No. 8,008,006 and European Pat. No. 1341808.

[d] U.S. Pat. No. 7,728,118.

[e] U.S. Pat. No. 7,906,282 and European Pat. No. 1341808.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega catalog online for the most up-to-date information on Promega products.