Technical Bulletin

pRL *Renilla* Luciferase Reporter Vectors

INSTRUCTIONS FOR USE OF PRODUCTS E2231, E2241, E2261 AND E2271.

www.promega.com

PRINTED IN USA.
12/08

AF9TB550 1208TB550

Part# TB550

pRL *Renilla* Luciferase Reporter Vectors

All technical literature is available on the Internet at: www.promega.com/tbs/ Please visit the web site to verify that you are using the most current version of this Technical Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com.

1. Description 1

2. Product Components and Storage Conditions 2

3. Features of the pRL Reporter Vectors 2
- A. The pRL Reporter Vectors 2
- B. Chimeric Intron 2
- C. T7 Promoter 3
- D. *Renilla* Luciferase Reporter Gene (*Rluc*) 3
- E. SV40 Late Polyadenylation Signal 4

4. Transfection of Mammalian Cells with the pRL Reporter Vectors 4

5. Related Products 6

6. References 7

1. Description

The pRL Reporter Vectors contain a cDNA (*Rluc*) encoding *Renilla* luciferase, which was originally cloned from the marine organism *Renilla reniformis* (sea pansy; 1). As described below, the *Renilla* luciferase cDNA contained within the pRL Reporter Vectors has been modified slightly to provide greater utility.

Renilla luciferase is a 36kDa monomeric protein that does not require post-translational modification for activity (2). Therefore, like firefly luciferase, the enzyme may function as a genetic reporter immediately following translation. For information about the use of this plasmid in conjunction with a reporter vector containing the firefly luciferase gene, refer to the *Dual-Luciferase® Reporter Assay System Technical Manual* (#TM040) or the *Dual-Glo® Luciferase Assay System Technical Manual* (#TM058).

To avoid DNA methylation, all pRL Reporter Vectors are isolated from a *dam*$^-$/*dcm*$^-$ *E. coli* K host strain. If you use methylation-sensitive restriction enzymes (e.g., BclI, ClaI, MboI, TaqI or XbaI), continue to propagate the pRL Reporter Vectors in a host strain with the same genetic background.

2. Product Components and Storage Conditions

Product	Size	Cat.#
pRL-SV40 Vector	20µg	E2231
pRL-TK Vector	20µg	E2241
pRL-CMV Vector	20µg	E2261
pRL-null Vector	20µg	E2271

All pRL Reporter Vectors are supplied in TE buffer (pH 7.4).

Storage Conditions: Store vector DNA at –20°C.

3. Features of the pRL Reporter Vectors

3.A. The pRL Reporter Vectors

The pRL-SV40 Vector[a] is intended for use as an internal control reporter vector and may be used in combination with any experimental reporter vector to co-transfect mammalian cells. The pRL-SV40 Vector contains the SV40 enhancer and early promoter elements to provide high-level expression of *Renilla* luciferase in co-transfected mammalian cells.

The pRL-TK Vector[a] is intended for use as an internal control reporter vector and may be used in combination with any experimental reporter vector to co-transfect mammalian cells. The pRL-TK Vector contains the herpes simplex virus thymidine kinase (HSV-TK) promoter to provide low to moderate levels of *Renilla* luciferase expression in co-transfected mammalian cells.

The pRL-CMV Vector[a,b] is intended for use as an internal control reporter vector and may be used in combination with any experimental reporter vector to co-transfect mammalian cells. The pRL-CMV Vector contains the CMV enhancer and immediate/early promoter elements to provide high-level expression of *Renilla* luciferase in co-transfected mammalian cells.

The pRL-null Vector[a] is intended for use in constructing a control reporter vector that may be used in combination with any experimental reporter vector to co-transfect mammalian cells. The pRL-null Vector contains no enhancer or promoter elements. Rather, it contains a multiple cloning region upstream of *Rluc* to allow for the cloning of any desired regulatory element(s) to drive expression of *Renilla* luciferase.

3.B. Chimeric Intron

Downstream of either the constitutive promoter regions (CMV, TK or SV40) or the multiple cloning site of the pRL Vectors is a chimeric intron comprised of the 5´-donor splice site from the first intron of the human β-globin gene, and the branch and 3´-acceptor splice site from an intron preceding an immunoglobulin gene heavy chain variable region (3). The sequences of the

donor and acceptor splice sites, along with the branchpoint site, have been modified to match the consensus sequences for optimal splicing (4).

Transfection studies have demonstrated that the presence of an intron flanking a cDNA insert frequently increases the level of gene expression (5–8). In the pRL Reporter Vectors, the intron is positioned 5´ to *Rluc* to minimize the utilization of cryptic 5´-donor splice sites that may reside within the reporter gene sequence (9).

3.C. T7 Promoter

A T7 promoter is located downstream of the chimeric intron and immediately precedes the *Rluc* reporter gene. This T7 promoter can be used to synthesize RNA transcripts in vitro using T7 RNA Polymerase (Cat.# P2075). T7 RNA Polymerase can also be used to synthesize active *Renilla* luciferase in a cell-free coupled eukaryotic in vitro transcription/translation reaction (e.g., our TNT® T7 Coupled Reticulocyte Lysate [Cat.# L4610], TNT® T7 Coupled Wheat Germ Extract [Cat.# L4140] or TNT® T7 Quick Coupled Transcription/Translation [Cat.# L1170] Systems).

Note: The T7 Promoter Primer offered by Promega (Cat.# Q5021) cannot be used for sequencing this vector because of a mismatch between the 3´ end of the primer and the vector DNA.

3.D. *Renilla* Luciferase Reporter Gene (*Rluc*)

The *Renilla* luciferase cDNA inserted into all of the pRL Reporter Vectors is derived from the anthozoan coelenterate *Renilla reniformis* (1) but contains nucleotide changes that were engineered during the construction of the individual vectors. The bases that were altered in each of the pRL Reporter Vectors are as follows:

pRL-CMV Vector	base 1298 (T→C) to eliminate an internal BglII site, base 1841 (T→C) to eliminate an internal BamHI site, base 1874 (C→T) to eliminate internal NarI, KasI, BanI and AcyI sites.
pRL-TK Vector	base 1264 (T→C) to eliminate an internal BglII site, base 1807 (T→C) to eliminate internal BamHI site, base 1840 (C→T) to eliminate internal NarI, KasI, BanI and AcyI sites.
pRL-null Vector	base 539 (T→C) to eliminate an internal BglII site, base 1082 (T→C) to eliminate an internal BamHI site, base 1115 (C→T) to eliminate internal NarI, KasI, BanI and AcyI sites.
pRL-SV40 Vector	base 924 (T→C) to eliminate an internal BamHI site, base 1500 (C→T) to eliminate internal NarI, KasI, BanI and AcyI sites.

These nucleotide substitutions do not alter the amino acid sequence of the encoded *Renilla* luciferase reporter enzyme.

3.E. SV40 Late Polyadenylation Signal

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 (10). Polyadenylation has been shown to enhance RNA stability and translation (11,12). The late SV40 polyadenylation signal is extremely efficient and has been shown to increase the steady-state level of RNA approximately fivefold more than the early SV40 polyadenylation signal (13). This poly(A) signal has been positioned 3´ to the *Rluc* gene in the pRL Reporter Vectors to increase the level of *Renilla* luciferase expression.

4. Transfection of Mammalian Cells with the pRL Reporter Vectors

The pRL-CMV, pRL-SV40, pRL-TK and pRL-null (once it has been modified to contain appropriate genetic regulatory domains) Vectors may be used in combination with any experimental reporter vector to co-transfect mammalian cells. However, it is important to realize that *trans* effects between promoters on co-transfected plasmids can potentially affect reporter gene expression (14). Primarily, this is of concern when either the control or experimental reporter vector, or both, contain very strong promoter/enhancer elements (such as CMV). The occurrence and magnitude of such effects will depend on several factors: a) the combination and activities of the genetic regulatory elements present on the co-transfected vectors, b) the relative ratio of experimental vector to control vector introduced into the cells, and c) the cell type transfected.

To help ensure independent genetic expression between experimental and control reporter genes, preliminary co-transfection experiments should be performed to optimize both the **amount** of vector DNA and the **ratio** of the co-reporter vectors added to the transfection mixture. Similar to the firefly luciferase assay, the *Renilla* luciferase assay is extremely sensitive, providing accurate measurement of ≤10 femtograms of *Renilla* luciferase, with linearity over 7 orders of enzyme concentration. Therefore, it is possible to use relatively small quantities of pRL-CMV, pRL-SV40 and pRL-TK Vectors to provide low-level, constitutive coexpression of *Renilla* luciferase control activity. Ratios of 10:1 to 50:1 (or greater) for experimental vector:pRL-CMV or pRL-SV40 Vector or 10:1 or greater for experimental vector:pRL-TK Vector combinations are feasible and may aid greatly in suppressing the occurrence of *trans* effects between promoter elements.

The pRL Reporter Vectors, including pRL-null once genetic regulatory domains have been added, can be used for both transient and stable expression of genes. For stable expression, the pRL Reporter Vectors must be co-transfected with an expression vector containing a selectable gene in mammalian cells. Transfection of DNA into mammalian cells may be mediated by cationic lipids (15,16), calcium phosphate (17,18), DEAE-dextran (19–21), polybrene-DMSO (22,23), or electroporation (24,25).

Transfection systems based on cationic lipid compounds (TransFast™ Transfection Reagent, and Tfx™-20 and Tfx™-50 Reagents), and calcium phosphate are available from Promega. Information about the TransFast™ Transfection Reagent can be found in the *TransFast™ Transfection Reagent Technical Bulletin* (#TB260). Protocols for the use of the Tfx™ Reagents can be found in the *Tfx™-20 and Tfx™-50 Reagents for the Transfection of Eukaryotic Cells Technical Bulletin* (#TB216). For transfection procedures using calcium phosphate, please request the *ProFection® Mammalian Transfection System Technical Manual* (#TM012).

Note: For assistance in determining transfection conditions for different cell lines, the Transfection Assistant is available online at:
www.promega.com/transfectionasst/

5. Related Products

pGL4 Luciferase Reporter Vectors

Please visit **www.promega.com/vectors/** to see a complete listing of our reporter vectors.

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat.#
pGL4.10[*luc2*]	Yes	*luc2*[A]	No	No	No	E6651
pGL4.11[*luc2P*]	Yes	"	hPEST	No	No	E6661
pGL4.12[*luc2CP*]	Yes	"	hCL1-hPEST	No	No	E6671
pGL4.13[*luc2*/SV40]	No	"	No	SV40	No	E6681
pGL4.14[*luc2*/Hygro]	Yes	"	No	No	Hygro	E6691
pGL4.15[*luc2P*/Hygro]	Yes	"	hPEST	No	Hygro	E6701
pGL4.16[*luc2CP*/Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6711
pGL4.17[*luc2*/Neo]	Yes	"	No	No	Neo	E6721
pGL4.18[*luc2P*/Neo]	Yes	"	hPEST	No	Neo	E6731
pGL4.19[*luc2CP*/Neo]	Yes	"	hCL1-hPEST	No	Neo	E6741
pGL4.20[*luc2*/Puro]	Yes	"	No	No	Puro	E6751
pGL4.21[*luc2P*/Puro]	Yes	"	hPEST	No	Puro	E6761
pGL4.22[*luc2CP*/Puro]	Yes	"	hCL1-hPEST	No	Puro	E6771
pGL4.70[*hRluc*]	Yes	*hRluc*[B]	No	No	No	E6881
pGL4.71[*hRlucP*]	Yes	"	hPEST	No	No	E6891
pGL4.72[*hRlucCP*]	Yes	"	hCL1-hPEST	No	No	E6901
pGL4.73[*hRluc*/SV40]	No	"	No	SV40	No	E6911
pGL4.74[*hRluc*/TK]	No	"	No	HSV-TK	No	E6921
pGL4.75[*hRluc*/CMV]	No	"	No	CMV	No	E6931
pGL4.76[*hRluc*/Hygro]	Yes	"	No	No	Hygro	E6941
pGL4.77[*hRlucP*/Hygro]	Yes	"	hPEST	No	Hygro	E6951
pGL4.78[*hRlucCP*/Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6961
pGL4.79[*hRluc*/Neo]	Yes	"	No	No	Neo	E6971
pGL4.80[*hRlucP*/Neo]	Yes	"	hPEST	No	Neo	E6981
pGL4.81[*hRlucCP*/Neo]	Yes	"	hCL1-hPEST	No	Neo	E6991
pGL4.82[*hRluc*/Puro]	Yes	"	No	No	Puro	E7501
pGL4.83[*hRlucP*/Puro]	Yes	"	hPEST	No	Puro	E7511
pGL4.84[*hRlucCP*/Puro]	Yes	"	hCL1-hPEST	No	Puro	E7521

[A]*luc2* = synthetic firefly luciferase gene. [B]*hRluc* = synthetic *Renilla* luciferase gene.

Luciferase Assay Systems

Product	Size	Cat.#
Renilla Luciferase Assay System	100 assays	E2810
	1,000 assays	E2820

Luciferase Assay Systems (continued)

Product	Size	Cat.#
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter Assay 10-Pack	1,000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System	1,000 assays	E1980

Product	Size	Cat.#
Dual-Glo® Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
EnduRen™ Live Cell Substrate	0.34mg	E6481
	3.4mg	E6482
	34mg	E6485
ViviRen™ Live Cell Substrate	0.37mg	E6491
	3.7mg	E6492
	37mg	E6495
GloMax®-Multi Detection System	1 each	E7031
GloMax® 96 Microplate Luminometer	1 each	E6501
GloMax® 20/20 Luminometer	1 each	E5311

6. References

1. Lorenz, W.W. *et al.* (1991) Isolation and expression of a cDNA encoding *Renilla reniformis* luciferase. *Proc. Natl. Acad. Sci. USA* **88**, 4438–42.
2. Matthews, J.C. *et al.* (1977) Purification and properties of *Renilla reniformis* luciferase. *Biochemistry* **16**, 85–91.
3. Bothwell, A.L.M. *et al.* (1981) Heavy chain variable region contribution to the NPb family of antibodies: Somatic mutation evident in a gamma 2a variable region. *Cell* **24**, 625–37.
4. Senapathy, P., Shapiro, M.B. and Harris, N.L. (1990) Splice junctions, branch point sites, and exons: Sequence statistics, identification, and applications to genome project. *Methods Enzymol.* **183**, 252–78.
5. Gross, M.K., Kainz, M.S. and Merrill, G.F. (1987) Introns are inconsequential to efficient formation of cellular thymidine kinase mRNA in mouse L cells. *Mol. Cell. Biol.* **7**, 4576–81.
6. Buchman, A.R. and Berg, P. (1988) Comparison of intron-dependent and intron-independent gene expression. *Mol. Cell. Biol.* **8**, 4395–405.
7. Evans, M.J. and Scarpulla, R.C. (1989) Introns in the 3′-untranslated region can inhibit chimeric CAT and beta-galactosidase gene expression. *Gene* **84**, 135–42.

6. References (continued)

8. Huang, M.T.F. and Gorman, C.M. (1990) Intervening sequences increase efficiency of RNA 3´ processing and accumulation of cytoplasmic RNA. *Nucleic Acids Res.* **18**, 937–47.

9. Huang, M.T.F. and Gorman, C.M. (1990) The simian virus 40 small-t intron, present in many common expression vectors, leads to aberrant splicing. *Mol. Cell. Biol.* **10**, 1805–10.

10. Proudfoot, N.J. (1991) Poly(A) signals. *Cell* **64**, 671–4.

11. Bernstein, P. and Ross, J. (1989) Poly(A), poly(A) binding protein and the regulation of mRNA stability. *Trends Biochem. Sci.* **14**, 373–7.

12. Jackson, R.J. and Standart, N. (1990) Do the poly(A) tail and 3´ untranslated region control mRNA translation? *Cell* **62**, 15–24.

13. Carswell, S. and Alwine, J.C. (1989) Efficiency of utilization of the simian virus 40 late polyadenylation site: Effects of upstream sequences. *Mol. Cell. Biol.* **9**, 4248–58.

14. Farr, A. and Roman, A. (1992) A pitfall of using a second plasmid to determine transfection efficiency. *Nucleic Acids Res.* **20**, 920.

15. Behr, J.P. *et al.* (1989) Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc. Natl. Acad. Sci. USA* **86**, 6982–6.

16. Loeffler, J.P. *et al.* (1990) Lipopolyamine-mediated transfection allows gene expression studies in primary neuronal cells. *J. Neurochem.* **54**, 1812–15.

17. Graham, F.L. and van der Eb, A.J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**, 456–67.

18. Wigler, M. *et al.* (1977) Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. *Cell* **11**, 223–32.

19. McCutchan, J.H. and Pagano, J.S. (1968) Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethylaminoethyl-dextran. *J. Natl. Cancer Inst.* **41**, 351–7.

20. Al-Moslih, M.I. and Dubes, G.R. (1973) The kinetics of DEAE-dextran-induced cell sensitization to transfection. *J. Gen. Virol.* **18**, 189–93.

21. Luthman, H. and Magnusson, G. (1983) High efficiency polyoma DNA transfection of chloroquine treated cells. *Nucleic Acids Res.* **11**, 1295–308.

22. Kawai, S. and Nishizawa, M. (1984) New procedure for DNA transfection with polycation and dimethyl sulfoxide. *Mol. Cell. Biol.* **4**, 1172–4.

23. Aubin, R.J., Weinfeld, M. and Paterson, M.C. (1988) Factors influencing efficiency and reproducibility of polybrene-assisted gene transfer. *Somat. Cell Mol. Genet.* **14**, 155–67.

24. Andreason, G.L. and Evans, G.A. (1988) Introduction and expression of DNA molecules in eukaryotic cells by electroporation. *BioTechniques* **6**, 650–60.

25. Neumann, E. *et al.* (1982) Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J.* **1**, 841–5.

[a]Licensed from University of Georgia Research Foundation, Inc., under U.S. Pat. Nos. 5,292,658, 5,418,155, Canadian Pat. No. 2,105,984 and related patents.

[b]The CMV promoter and its use are covered under U.S. Pat. Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation, Iowa City, Iowa, and licensed FOR RESEARCH USE ONLY. Research Use includes contract research for which monetary or other consideration may be received. Other commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation.

Dual-Glo, Dual-Luciferase, GloMax, ProFection and TNT are registered trademarks of Promega Corporation. EnduRen, Tfx, TransFast and ViviRen are trademarks of Promega Corporation.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

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