



Promega

Technical Bulletin

pRL-CMV Vector

INSTRUCTIONS FOR USE OF PRODUCT E2261.



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PRINTED IN USA.
Revised 10/07



AF9TE237 1007TE237

Part# TB237

pRL-CMV Vector

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I. Description

The pRL-CMV Vector^(a,b) (Figure 1) is intended for use as an internal control reporter and may be used in combination with any experimental reporter vector to co-transfect mammalian cells. All of 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 Vectors has been modified slightly to provide greater utility.

The pRL-CMV Vector contains the CMV enhancer and early promoter elements to provide high-level expression of *Renilla* luciferase in co-transfected mammalian cells. *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 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-CMV Vector in the same genetic background.

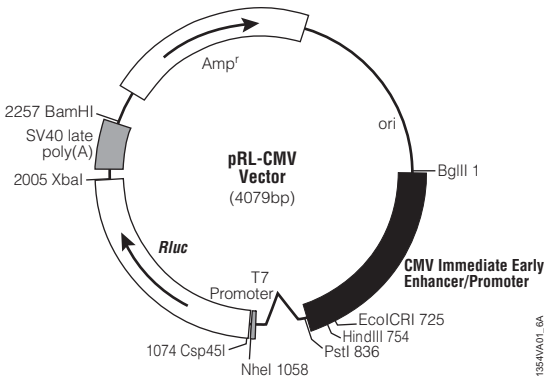
II. Product Components and Storage Conditions

Product	Size	Cat.#
pRL-CMV Vector	20µg	E2261

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

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

Figure 1. The pRL-CMV Vector circle map and sequence reference points.



Sequence reference points:

CMV enhancer and immediate early promoter	7-803
Chimeric intron	860-996
T7 promoter (-17 to +2)	1040-1058
T7 promoter transcription start site	1057
<i>Rluc</i> reporter gene	1068-2003
SV40 late polyadenylation signal	2045-2246
β -lactamase (<i>Amp^r</i>) coding region	2393-3253

Note: -[^]-, position of intron; *Rluc*, cDNA encoding the *Renilla* luciferase enzyme; *Amp^r*, gene conferring ampicillin resistance in *E. coli*; ori, origin of plasmid replication in *E. coli*. Arrows within the *Rluc* and *Amp^r* gene indicate the direction of transcription.

III. Features of the pRL-CMV Vector

III.A. CMV Enhancer/Promoter Regions

The pRL-CMV Vector contains the CMV immediate-early enhancer/promoter region, which provides strong, constitutive expression of the *Renilla* luciferase cDNA in a variety of cell types. The promiscuous nature of the CMV enhancer/promoter has been demonstrated in transgenic mice, where its transcriptional activity was observed in 24 of the 28 murine tissues examined (3).

III.B. Chimeric Intron

Downstream of the CMV enhancer/promoter region of the pRL-CMV Vector 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 (4). 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 (5).

Transfection studies have demonstrated that the presence of an intron flanking a cDNA insert frequently increases the level of gene expression (6-9). In the pRL-CMV Vector, 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 (10).

III.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.

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

The *Renilla* luciferase cDNA inserted into all of the pRL 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 following bases were altered in the pRL-CMV Vector: base 1298 (T→C) to eliminate an internal BglIII site, base 1841 (T→C) to eliminate an internal BamHI site, base 1874 (C→T) to eliminate internal NarI, KasI, BanI and AclI sites. These nucleotide substitutions do not alter the amino acid sequence of the encoded *Renilla* luciferase reporter enzyme.

III.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 (11). Polyadenylation has been shown to enhance RNA stability and translation (12,13). The late SV40 polyadenylation signal, which is extremely efficient and has been shown to increase the steady-state level of RNA approximately fivefold more than the early SV40 polyadenylation signal (14), has been positioned 3' to the *Rluc* gene in the pRL-CMV Vector to increase the level of *Renilla* luciferase expression.

IV. Transfection of Mammalian Cells with pRL-CMV Vector

The pRL-CMV Vector 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 (15). 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 Vector 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 Vector combinations are feasible and may aid greatly in suppressing the occurrence of *trans* effects between promoter elements.

The pRL-CMV Vector can be used for both transient and stable expression of genes. For stable expression, the pRL-CMV Vector 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 (16,17), calcium phosphate (18,19), DEAE-dextran (20–22), polybrene-DMSO (23,24), or electroporation (25,26).

Transfection systems based on cationic lipid compounds (Transfectam[®] Reagent, TransFast[™] Transfection Reagent, and Tfx[™]-20 and Tfx[™]-50

Reagents), and calcium phosphate are available from Promega. For more information and a protocol for the Transfectam® Reagent, please request the *Transfectam® Reagent for the Transfection of Eukaryotic Cells Technical Bulletin* (#TB116). 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, we offer the Transfection Assistant available online at:

www.promega.com/transfectionasst/

V. pRL-CMV Vector Restriction Sites

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3' end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch Office or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number **AF025843**) and on the Internet at: www.promega.com/vectors/

Table 1. Restriction Enzymes That Cut the pRL-CMV Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
AatII	4	284, 337, 420, 606	BamHI	1	2257
AcyI	5	281, 334, 417, 603, 2640	BanI	4	624, 949, 1872, 3227
AflII	2	826, 1023	BanII	1	727
AflIII	1	1250	BbsI	2	934, 1908
Alw44I	2	2508, 3754	BclI	2	1352, 1561
AlwNI	1	3659	BglI	5	142, 249, 371, 442, 3062
AspHI	4	727, 2512, 2597, 3758	BglII	1	1
AvaII	4	1116, 1838, 2816, 3038	BsaI	2	888, 3114
Ball	2	16, 70	BsaOI	4	2015, 2662, 2811, 3734
			BsaAI	2	499, 1800

Note: The enzymes listed in boldface type are available from Promega.

V. pRL-CMV Vector Restriction Sites (continued)

Table 1. Restriction Enzymes That Cut the pRL-CMV Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
BsaBI	1	2256	HincII	2	675, 2155
BsaHI	5	281, 334, 417, 603, 2640	HindII	2	675, 2155
BsaJI	3	519, 1841, 3908	HindIII	1	754
BsaMI	2	2076, 2169	HpaI	1	2155
BsmI	2	2076, 2169	Hsp92I	5	281, 334, 417, 603, 2640
Bsp1286I	4	727, 2512, 2597, 3758	MspAII	3	2544, 3485, 3730
BspHI	3	1636, 2340, 3348	NciI	3	2644, 2995, 3691
BspMI	1	850	NcoI	1	519
BsrGI	2	102, 1766	NdeI	1	393
BssSI	3	1726, 2511, 3895	NheI	1	1058
Bst98I	2	826, 1023	NotI	1	2012
BstZI	1	2012	NspI	2	1194, 1254
ClaI	1	2250	PleI	5	563, 924, 1040, 3189, 3692
Csp45I	1	1074	PstI	1	836
DdeI	4	2679, 3219, 3385, 3794	PvuI	1	2811
DraI	4	2216, 2602, 3294, 3313	SacI	1	727
DrdI	2	815, 3966	ScaI	2	1036, 2699
DsaI	1	519	SinI	4	1116, 1838, 2816, 3038
EaeI	5	14, 68, 1418, 2012, 2787	SnaBI	1	499
EagI	1	2012	SpeI	1	158
EarI	2	1238, 2381	SspI	3	11, 58, 2375
EclHKI	1	3180	StyI	1	519
Eco52I	1	2012	TfiI	5	1158, 1179, 1518, 1833, 1846
EcoICRI	1	725	VspI	3	166, 1168, 3005
FspI	1	2957	XbaI	1	2005
HaeII	1	3828	XcmI	1	1717
HgaI	4	685, 2648, 3378, 3956	XmnI	2	1602, 2580

Note: The enzymes listed in boldface type are available from Promega.

Table 2. Restriction Enzymes That Do Not Cut the pRL-CMV Vector.

Acc65I	BlnI	FseI	PaeR7I	SmaI
AccB7I	BssHII	I-PpoI	PmeI	SphI
AccI	BstEII	KasI	PmlI	StuI
AccIII	BstXI	KpnI	PpuMI	SwaI
AgeI	Bsu36I	MluI	PshAI	Tth111I
Apal	CspI	NaeI	PvuII	XhoI
AscI	DraIII	NarI	SacII	XmaI
AvaI	Eco47III	NgoMIV	SalI	
AvrII	EcoNI	NruI	SfiI	
BbeI	EcoRI	NsiI	SgfI	
BbuI	EcoRV	PacI	SgrAI	

Table 3. Restriction Enzymes That Cut the pRL-CMV Vector 6 or More Times.

AccI	BstUI	HinfI	MboII	Sau3AI
AluI	CfoI	HpaII	MnlI	Sau96I
Alw26I	DpnI	HphI	MseI	ScrFI
BbvI	DpnII	Hsp92II	MspI	SfaNI
BsrI	Fnu4HI	MaeI	NdeII	TaqI
BsrSI	FokI	MaeII	NlaIII	Tru9I
Bst7II	HaeIII	MaeIII	NlaIV	XhoII
BstOI	HhaI	MboI	RsaI	

VI. Related Products

pRL Family of *Renilla* Luciferase Vectors for Co-Reporter Applications

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

To inquire about the availability of bulk packaging and pricing for pRL Vectors, please contact Promega. For inquiries on the availability of new promoter variations within the pRL family of co-reporter vectors, contact Technical Services or visit our web site at: www.promega.com

VI. Related Products (continued)

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	<i>luc2</i> ^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	<i>hRluc</i> ^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.#
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

VI. Related Products (continued)

Luciferase Assay Systems (continued)

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

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