



Promega

Technical Bulletin

pRL-SV40 Vector

INSTRUCTIONS FOR USE OF PRODUCT E2231.



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pRL-SV40 Vector

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I. Description

The pRL-SV40 Vector^(a) (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 our 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-SV40 Vector contains the SV40 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*⁻/*dcn*⁻ *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-SV40 Vector in the same genetic background.

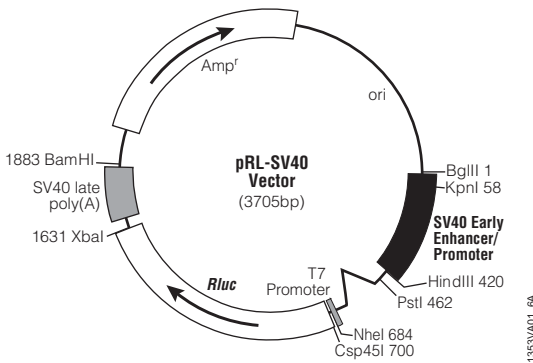
II. Product Components and Storage Conditions

Product	Size	Cat.#
pRL-SV40 Vector	20µg	E2231

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

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

Figure 1. The pRL-SV40 Vector circle map and sequence reference points.



Sequence reference points:

SV40 enhancer and early promoter	7-418
Chimeric intron	486-622
T7 RNA polymerase promoter (-17 to +2)	666-684
T7 RNA polymerase transcription initiation site	683
<i>Rluc</i> reporter gene	694-1629
SV40 late polyadenylation signal	1671-1872
β-lactamase (<i>Amp^r</i>) coding region	2019-2879

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-SV40 Vector

III.A. SV40 Enhancer/Promoter Regions

The pRL-SV40 Vector contains the SV40 enhancer/promoter region, which provides strong, constitutive expression of *Rluc* in a variety of cell types. The vectors also contains the SV40 origin of replication, which allows transient, episomal replication in cells expressing the SV40 large T antigen, such as COS-1 or COS-7 cells (3).

III.B. Chimeric Intron

Downstream of the SV40 enhancer/promoter region of the pRL-SV40 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-SV40 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, immediately preceding 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-SV40 Vector: base 924 (T→C) to eliminate an internal BamHI site, base 1500 (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-SV40 Vector to increase the level of *Renilla* luciferase expression.

IV. Transfection of Mammalian Cells with pRL-SV40

The pRL-SV40 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). This is primarily of concern when either the control or experimental reporter vector, or both, contain very strong promoter/enhancer elements. The occurrence and magnitude of such effects will depend on several factors: i) the combination and activities of the genetic regulatory elements present on the co-transfected vectors, ii) the relative ratio of experimental vector to control vector introduced into the cells, and iii) 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-SV40 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-SV40 Vector combinations are feasible and may aid greatly in suppressing the occurrence of *trans* effects between promoter elements.

The pRL-SV40 Vector can be used for both transient and stable expression of genes. For stable expression, the pRL-SV40 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-SV40 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 **AF025845**) and on the Internet at: www.promega.com/vectors/

Table 1. Restriction Enzymes That Cut the pRL-SV40 Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Acc65I	1	54	BanI	4	54, 575, 1498
AcyI	1	2266			2853
AflII	2	452, 649	BbsI	2	560, 1534
AflIII	1	876	BbuI	2	152, 224
Alw26I	5	449, 514, 539, 1964, 2740	BclI	2	978, 1187
Alw44I	2	2134, 3380	BglI	2	357, 2688
AlwNI	1	3285	BglII	1	1
AspHI	3	2138, 2223, 3384	BsaI	2	514, 2740
AvaII	4	742, 1464, 2442 2664	BsaOI	4	1641, 2288, 2437, 3360
AvrII	1	404	BsaAI	1	1426
BamHI	1	1883	BsaBI	1	1882
			BsaHI	1	2266
			BsaMI	2	1702, 1795

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

V. pRL-SV40 Vector Restriction Sites (continued)

Table 1. Restriction Enzymes That Cut the pRL-SV40 Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
BsmI	2	1702, 1795	MaeII	5	1388, 1425, 2204, 2577, 2993
Bsp1286I	3	2138, 2223, 3384	MspAII	4	80, 2170, 3111, 3356
BspFI	3	1262, 1966, 2974	NciI	3	2270, 2621, 3317
BspMI	1	476	NcoI	2	15, 311
BsrGI	1	1392	NheI	1	684
BssSI	3	1352, 2137, 3521	NotI	1	1638
Bst98I	2	452, 649	NsiI	2	154, 226
BstZI	1	1638	NspI	4	152, 224, 820, 880
Cfr10I	1	2721	PleI	4	550, 666, 2815, 3318
Clal	1	1876	Ppu10I	2	150, 222
Csp45I	1	700	PstI	1	462
DraI	4	1842, 2228, 2920, 2939	PvuI	1	2437
DrdI	2	441, 3592	PvuII	1	80
DsaI	2	15, 311	RsaI	4	56, 662, 1394, 2325
EaeI	3	1044, 1638, 2413	ScaI	2	662, 2325
EagI	1	1638	SfiI	1	357
EarI	2	864, 2007	SinI	4	742, 1464, 2442, 2664
EclHKI	1	2806	SphI	2	152, 224
Eco52I	1	1638	SspI	1	2001
FspI	2	8, 2583	StuI	1	403
HaeII	1	3454	StyI	3	15, 311, 404
HgaI	3	2274, 3004, 3582	VspI	2	794, 2631
HincII	1	1781	XbaI	1	1631
HindII	1	1781	XcmI	1	1343
HindIII	1	420	XmnI	2	1228, 2206
HpaI	1	1781			
Hsp92I	1	2266			
KpnI	1	58			

Table 2. Restriction Enzymes That Do Not Cut the pRL-SV40 Vector.

AatII	Bsp120I	EcoRI	PfiMI	SmaI
AccB7I	BssHII	EcoRV	PinAI	SnaBI
AccI	Bst1107I	EheI	PmeI	SpeI
AccIII	BstEII	FseI	PmlI	SpII
AgeI	BstXI	I-PpoI	PpuMI	SrfI
Apal	Bsu36I	KasI	PshAI	Sse8387I
AscI	CspI	MluI	Psp5II	SwaI
AvaI	DraII	NaeI	PspAI	Tth111I
BalI	DraIII	NarI	RsrII	XhoI
BanII	Eco47III	NdeI	SacI	XmaI
BbeI	Eco72I	NgoMIV	SacII	
BbrPI	Eco81I	NruI	SalI	
BlpI	EcoICRI	Pacl	SgfI	
Bpu1102I	EcoNI	PaeR7I	SgrAI	

Table 3. Restriction Enzymes That Cut the pRL-SV40 Vector 6 or More Times.

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

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

VI. Related Products

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

Product	Size	Cat.#
pRL-TK Vector	20µg	E2241
pRL-CMV Vector	20µg	E2261
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

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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