



**Promega**

# Technical Bulletin

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## **pRL-TK Vector**

INSTRUCTIONS FOR USE OF PRODUCT E2241.



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Part# TB240

# pRL-TK Vector

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## I. Description

The pRL-TK Vector<sup>(a)</sup> (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-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. *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*<sup>-</sup>/*dcm*<sup>-</sup> *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-TK Vector in the same genetic background.

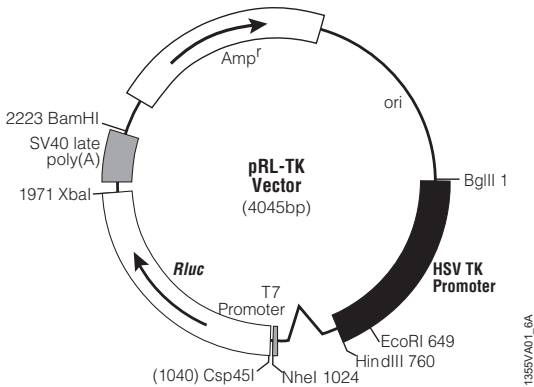
## II. Product Components and Storage Conditions

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

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

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

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



### Sequence reference points:

HSV-TK promoter	7-759
Chimeric intron	826-962
T7 RNA polymerase promoter (-17 to +2)	1006-1024
T7 RNA polymerase transcription initiation site	1023
<i>Rluc</i> reporter gene	1034-1969
SV40 late polyadenylation signal	2011-2212
β-lactamase ( <i>Amp<sup>r</sup></i> ) coding region	2359-3219

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

Restriction sites shown in parentheses are **not** unique sites.

### III. Features of the pRL-TK Vector

#### III.A. TK Promoter Region

The pRL-TK Vector contains the herpes simplex virus thymidine kinase promoter region upstream of *Rluc*. The HSV-TK promoter provides low-level, constitutive expression in cells of both embryonal and mature mammalian tissues (3,4).

#### III.B. Chimeric Intron

Downstream of the TK promoter region of the pRL-TK Vector is a chimeric intron comprised of the 5'-donor splice site from the first intron of the human  $\beta$ -globin gene, and the branch and 3'-acceptor splice site from an intron preceding an immunoglobulin gene heavy chain variable region (5). 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 (6).

Transfection studies have demonstrated that the presence of an intron flanking a cDNA insert frequently increases the level of gene expression (7-10). 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 (11).

#### 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<sup>®</sup> T7 Coupled Reticulocyte Lysate [Cat.# L4610], TNT<sup>®</sup> T7 Coupled Wheat Germ Extract [Cat.# L4140] or TNT<sup>®</sup> 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-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. 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 (12). Polyadenylation has been shown to enhance RNA stability and translation (13,14). 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 (15), has been positioned 3' to the *Rluc* gene in the pRL-TK Vector to increase the level of *Renilla* luciferase expression.

### IV. Transfection of Mammalian Cells with pRL-TK

The pRL-TK 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 (16). 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 cotransfected vectors, ii) the relative ratio of experimental vector to control vector introduced into the cells, and iii) the type of cell 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 coreporter vectors added to the transfection mixture. Similar to firefly luciferase, *Renilla* luciferase is extremely sensitive, providing accurate measurement of  $\leq 10$  femtograms, with linearity over 7 orders of enzyme concentration. Therefore, it may be possible to use relatively small quantities of pRL-TK Vector to provide low-level, constitutive coexpression of *Renilla* luciferase control activity. Ratios of 10:1 (or greater) for experimental vector:pRL-TK Vector combinations may aid greatly in suppressing the occurrence of *trans* effects between promoter elements.

The pRL-TK Vector can be used for both transient and stable expression of genes. For stable expression, the pRL-TK 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 (17,18), calcium phosphate (19,20), DEAE-dextran (21–23), polybrene-DMSO (24,25) or electroporation (26,27).

Transfection systems based on cationic lipid compounds (Transfectam<sup>®</sup> Reagent, TransFast<sup>™</sup> Transfection Reagent, and Tfx<sup>™</sup>-20 and Tfx<sup>™</sup>-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/](http://www.promega.com/transfectionasst/)

## V. pRL-TK 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 **AF025846**) and on the Internet at: [www.promega.com/vectors/](http://www.promega.com/vectors/)

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

<b>Enzyme</b>	<b># of Sites</b>	<b>Location</b>	<b>Enzyme</b>	<b># of Sites</b>	<b>Location</b>
<b>AccI</b>	1	342	<b>BclI</b>	2	1318, 1527
AcyI	2	290, 2606	<b>BglI</b>	3	298, 301, 3028
AflII	4	36, 160, 792, 989	<b>BglIII</b>	1	1
AflIII	2	713, 1216	BsaI	3	386, 854, 3080
<b>Alw44I</b>	2	2474, 3720	BsaOI	4	1981, 2628, 2777, 3700
AlwNI	2	357, 3625	BsaAI	2	346, 1766
AspHI	3	2478, 2563, 3724	BsaBI	1	2222
<b>AvaI</b>	3	108, 229, 282	BsaHI	2	290, 2606
AvrII	1	322	<b>BsaMI</b>	2	2042, 2135
<b>BalI</b>	1	128	BsmI	2	2042, 2135
<b>BamHI</b>	1	2223	BspHI	3	1602, 2306, 3314
<b>BanI</b>	4	289, 915, 1838, 3193	BspMI	1	816
<b>BanII</b>	1	313	BsrGI	1	1732
BbeI	1	293	<b>BssHII</b>	1	241
BbsI	3	6, 900, 1874	BssSI	3	1692, 2477, 3861

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

V. pRL-TK Vector Restriction Sites (continued)

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

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Bst1107I	1	343	HpaI	1	2121
<b>Bst98I</b>	4	36, 160, 792, 989	<b>Hsp92I</b>	2	290, 2606
<b>BstZI</b>	1	1978	KasI	1	289
Cfr10I	1	3061	<b>MluI</b>	1	713
<b>ClaI</b>	1	2216	<b>NarI</b>	1	290
<b>Csp45I</b>	2	653, 1040	<b>NcoI</b>	1	330
<b>DdeI</b>	4	2645, 3185, 3351, 3760	<b>NheI</b>	1	1024
<b>DraI</b>	4	2182, 2568, 3260, 3279	<b>NotI</b>	1	1978
DraII	1	319	NspI	3	599, 1160, 1220
DrdI	2	781, 3932	PpuMI	1	319
DsaI	5	129, 180, 296, 330, 543	Psp5II	1	319
EaeI	4	126, 1384, 1978, 2753	PspAI	1	282
EagI	1	1978	<b>PstI</b>	3	509, 746, 802
EarI	3	137, 1204, 2347	<b>PvuI</b>	1	2777
<b>EclHKI</b>	1	3146	<b>PvuII</b>	1	531
Eco52I	1	1978	<b>RsaI</b>	4	473, 1002, 1734, 2665
<b>EcoRI</b>	1	649	<b>SacII</b>	1	299
EheI	1	291	<b>ScaI</b>	2	1002, 2665
FspI	1	2923	<b>SmaI</b>	1	284
<b>HaeII</b>	3	150, 293, 3794	<b>SspI</b>	1	2341
<b>HincII</b>	1	2121	<b>StyI</b>	2	322, 330
HindII	1	2121	<b>VspI</b>	2	1134, 2971
<b>HindIII</b>	1	760	<b>XbaI</b>	1	1971
			XcmI	1	1683
			<b>XmaI</b>	1	282
			<b>XmnI</b>	2	1568, 2546

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

AatII	Bsp120I	<b>EcoRV</b>	PfIMI	SgrAI
<b>AccB7I</b>	<b>BstEII</b>	FseI	PinAI	<b>SnaBI</b>
<b>AccIII</b>	<b>BstXI</b>	<b>I-PpoI</b>	PmeI	<b>SpeI</b>
<b>Acc65I</b>	<b>Bsu36I</b>	<b>KpnI</b>	PmlI	<b>SphI</b>
<b>AgeI</b>	<b>CspI</b>	<b>NaeI</b>	Ppu10I	SplI
<b>ApaI</b>	DraIII	<b>NdeI</b>	PshAI	SrfI
AscI	<b>Eco47III</b>	<b>NgoMIV</b>	RsrII	Sse8387I
BbrPI	Eco72I	<b>NruI</b>	<b>SacI</b>	<b>StuI</b>
<b>BbuI</b>	Eco81I	<b>NsiI</b>	<b>SalI</b>	Swal
BlpI	<b>EcoICRI</b>	PacI	<b>SfiI</b>	<b>Tth111I</b>
Bpu1102I	EcoNI	PaeR7I	<b>SgfI</b>	<b>XhoI</b>

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

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

AcI	<b>BstOI</b>	<b>HinI</b>	MseI20	ScrFI
<b>AluI</b>	BstUI	<b>HpaII</b>	<b>MspI</b>	SfaNI
<b>Alw26I</b>	<b>CfoI</b>	HphI	<b>MspAII</b>	<b>SinI</b>
<b>AvaII</b>	<b>DpnI</b>	<b>Hsp92II</b>	NciI	<b>TaqI</b>
BbvI	DpnII	MaeI	<b>NdeII</b>	TfiI
BsaJI	Fnu4HI	MaeII	NlaIII	<b>Tru9I</b>
<b>Bsp1286I</b>	<b>FokI</b>	MaeIII	NlaIV	<b>XhoII</b>
BsrI	<b>HaeIII</b>	MboI	PleI	
<b>BsrSI</b>	HgaI	<b>MboII</b>	<b>Sau3AI</b>	
Bst7II	<b>HhaI</b>	MnI	Sau96I	

**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-CMV Vector	20µg	E2261
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](http://www.promega.com)

### 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
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

## VI. Related Products (continued)

### pGL4 Luciferase Reporter Vectors

Please visit [www.promega.com/vectors/](http://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[ <i>luc2</i> ]	Yes	<i>luc2</i> <sup>A</sup>	No	No	No	E6651
pGL4.11[ <i>luc2P</i> ]	Yes	"	hPEST	No	No	E6661
pGL4.12[ <i>luc2CP</i> ]	Yes	"	hCL1-hPEST	No	No	E6671
pGL4.13[ <i>luc2</i> /SV40]	No	"	No	SV40	No	E6681
pGL4.14[ <i>luc2</i> /Hygro]	Yes	"	No	No	Hygro	E6691
pGL4.15[ <i>luc2P</i> /Hygro]	Yes	"	hPEST	No	Hygro	E6701
pGL4.16[ <i>luc2CP</i> /Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6711
pGL4.17[ <i>luc2</i> /Neo]	Yes	"	No	No	Neo	E6721
pGL4.18[ <i>luc2P</i> /Neo]	Yes	"	hPEST	No	Neo	E6731
pGL4.19[ <i>luc2CP</i> /Neo]	Yes	"	hCL1-hPEST	No	Neo	E6741
pGL4.20[ <i>luc2</i> /Puro]	Yes	"	No	No	Puro	E6751
pGL4.21[ <i>luc2P</i> /Puro]	Yes	"	hPEST	No	Puro	E6761
pGL4.22[ <i>luc2CP</i> /Puro]	Yes	"	hCL1-hPEST	No	Puro	E6771
pGL4.70[ <i>hRluc</i> ]	Yes	<i>hRluc</i> <sup>B</sup>	No	No	No	E6881
pGL4.71[ <i>hRlucP</i> ]	Yes	"	hPEST	No	No	E6891
pGL4.72[ <i>hRlucCP</i> ]	Yes	"	hCL1-hPEST	No	No	E6901
pGL4.73[ <i>hRluc</i> /SV40]	No	"	No	SV40	No	E6911
pGL4.74[ <i>hRluc</i> /TK]	No	"	No	HSV-TK	No	E6921
pGL4.75[ <i>hRluc</i> /CMV]	No	"	No	CMV	No	E6931
pGL4.76[ <i>hRluc</i> /Hygro]	Yes	"	No	No	Hygro	E6941
pGL4.77[ <i>hRlucP</i> /Hygro]	Yes	"	hPEST	No	Hygro	E6951
pGL4.78[ <i>hRlucCP</i> /Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6961
pGL4.79[ <i>hRluc</i> /Neo]	Yes	"	No	No	Neo	E6971
pGL4.80[ <i>hRlucP</i> /Neo]	Yes	"	hPEST	No	Neo	E6981
pGL4.81[ <i>hRlucCP</i> /Neo]	Yes	"	hCL1-hPEST	No	Neo	E6991
pGL4.82[ <i>hRluc</i> /Puro]	Yes	"	No	No	Puro	E7501
pGL4.83[ <i>hRlucP</i> /Puro]	Yes	"	hPEST	No	Puro	E7511
pGL4.84[ <i>hRlucCP</i> /Puro]	Yes	"	hCL1-hPEST	No	Puro	E7521

<sup>A</sup>*luc2* = synthetic firefly luciferase gene. <sup>B</sup>*hRluc* = synthetic *Renilla* luciferase gene.

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