Technical Bulletin

pSV-β-Galactosidase Control Vector

INSTRUCTIONS FOR USE OF PRODUCT E1081.

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pSV-β-Galactosidase Control Vector

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I. Description

The pSV-β-Galactosidase Control Vector is designed as a positive control vector for monitoring transfection efficiencies of mammalian cells. The SV40 early promoter and enhancer drive transcription of the bacterial *lac*Z gene, which in turn, is translated into the β-galactosidase enzyme. β-galactosidase is an excellent reporter enzyme (1,2) that can be assayed quickly and directly in cell extracts using spectrophotometric, fluorescent or chemiluminescent assays (3,4). This reporter enzyme is also widely used for in situ histochemical analysis using the substrate X-Gal (5).

The pSV-β-Galactosidase Control Vector can be co-transfected with your DNA of interest. For example, co-transfection with firefly luciferase gene vectors provide cell extracts that can be assayed for both luciferase and β-galactosidase activities. In this manner, the pSV-β-Galactosidase Vector acts as an internal control for transient expression assays. A negative control extract, prepared from mock-transfected cells, should also be assayed for the presence of endogenous β-galactosidase activity in cultured cells (2). In addition, co-transfection with chloramphenicol acetyltransferse reporter gene vectors (e.g., pCAT®3 Vectors) permits assaying for both CAT and β-galactosidase activities.

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I. Description (continued)

The pSV-β-Galactosidase Vector is a modification of pRSV-βGAL (6) with SV40 and pUC18 sequences substituted for RSV and pBR322 sequences. The pSV-β-Galactosidase Vector will express β-galactosidase in *E. coli* due to the presence of the *E. coli gpt* promoter located upstream of the *lacZ* gene (1). Colonies of *E. coli* containing the pSV-β-Galactosidase Vector will appear blue when plated on media containing X-gal.

II. Product Components and Storage Conditions

Product	Size	Cat.#
pSV-β-Galactosidase Control Vector	20µg	E1081

Storage Conditions: Store the pSV-β-Galactosidase Control Vector at –20°C.

III. General Usage Considerations

The recommended amount of pSV-β-Galactosidase Control Vector to use for transfection of cells (60 or 100mm dish) is 5–10µg. The optimal amount of plasmid DNA will be determined by the efficiency of transfection, which is dependent upon the particular cell line and transfection protocol.

Several methods can be used to prepare cell extracts to be assayed for β-galactosidase activity. Reporter Lysis Buffer, supplied with the β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer (Cat.# E2000), or available separately (Cat.# E3971) allows β-galactosidase, chloramphenicol acetyltransferase (CAT) and luciferase assays to be performed from the same cell extract. In cell lines that we have tested, β-galactosidase activity in the Reporter Lysis Buffer is significantly higher than activity in extracts prepared with the freeze-thaw method. Complete instructions are supplied with the buffer.

For β-galactosidase assays not using the Reporter Lysis Buffer, we recommend the protocols found in the *Protocols and Applications Guide* chapter on Bioluminescence Reporters (7).

IV. pSV-β-Galactosidase Control Vector Circle Map

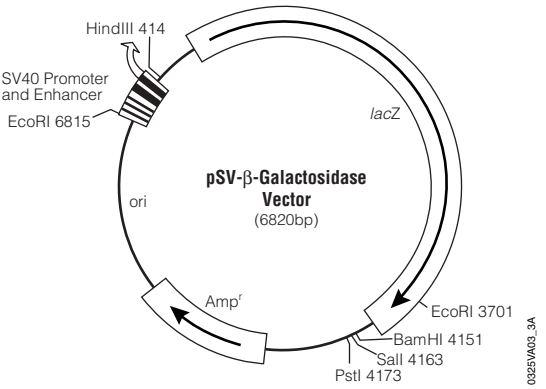

Figure 1. pSV-β-Galactosidase Control Vector circle map and sequence reference points.

Sequence reference points:

SV40 early promoter and enhancer segment	1–419
Transcription start sites	354, 360, 365
gpt promoter (–10 region)	428–433
Possible start codons (ATG)	500, 530, 569
operon sequences	709–4020
*lac*Z start site	710
*lac*Z stop site (TAA)	3755
*lac*Y	3809–4011
SV40 small T antigen	4021–4156
β-lactamase (Amp^r) coding region	4784–5644

Note: The *lac*Z coding region in this vector starts with the 7th amino acid of the wildtype *lac*Z gene.

V. pSV-β-Galactosidase Control 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 or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are available in the GenBank® database (GenBank®/EMBL Accession Number **X65335**) and on the Internet at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pSV-β-Galactosidase Control Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	2	1320, 4652
AccB7I	2	1942, 2308
AccI	2	3461, 4164
Acc65I	2	48, 619
AgeI	1	622
Alw44I	5	3042, 3157, 4402, 4899, 6145
AlwNI	4	2407, 2832, 3583, 6050
AvaI	2	2035, 3478
AvaII	3	2238, 5207, 5429
AvrII	1	398
BamHI	1	4151
BanII	2	2637, 3686
BbeI	1	4348
BbsI	3	639, 2214, 3466
BbuI	3	146, 218, 4179
BclI	1	2043
BglI	5	351, 849, 2970, 4335, 5453
BlpI	1	3710
Bpu1102I	1	3710
BsaI	1	5505
BsaAI	1	548
BsaBI	1	2028
BsaMI	1	4101
BsmI	1	4101
BspHI	3	4626, 4731, 5739
BspMI	3	1471, 3343, 4176
BssHII	1	2195
BssSI	4	1754, 4595, 4902, 6286
Bst1107I	1	3462
BstXI	2	2917, 3534
Bsu36I	1	921
Cfr10I	4	622, 1984, 3716, 5486
ClaI	2	702, 1521
DraI	4	3819, 4993, 5685, 5704
DraII	1	4591
DraIII	1	1887
DrdI	3	2806, 4488, 6357
EaeI	5	1168, 2166, 4191, 5178, 6620
EarI	4	798, 4284, 4772, 6576
EclHKI	1	5571
Eco47III	1	2532
Eco81I	1	921
EcoICRI	1	2635
EcoRI	2	3701, 6814
EcoRV	1	1810
EheI	1	4346
FspI	3	839, 4325, 5348
HincII	4	1123, 1747, 3575, 4165

Table 1. Restriction Enzymes That Cut the pSV-β-Galactosidase Control Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
HindII	4	1123, 1747, 3575, 4165	**PstI**	1	4173
			SacI	1	2637
HindIII	1	414	**SalI**	1	4163
HpaI	2	1123, 1747	**ScaI**	1	5090
KasI	1	4344	**SfiI**	1	351
KpnI	2	52, 623	**SinI**	3	2238, 5207, 5429
MluI	3	1999, 2779, 3204	**SphI**	3	146, 218, 4179
NarI	1	4345	SplI	1	3468
NcoI	2	9, 305	Sse8387I	1	4173
NdeI	2	3654, 4397	**SspI**	2	1927, 4766
NsiI	2	148, 220	**StuI**	1	397
PflMI	2	1942, 2308	**StyI**	3	9, 305, 398
PinAI	1	622	**VspI**	3	5396, 6631, 6690
PleI	5	3668, 4169, 5580, 6083, 6568	**XbaI**	1	4157
			XcmI	3	2914, 3659, 3992
Ppu10I	2	144, 216	**XmnI**	1	4971

Table 2. Restriction Enzymes That Do Not Cut the pSV-β-Galactosidase Control Vector.

AccIII	**Bst98I**	FseI	PmeI	SgrAI
AflII	**BstEII**	**I-PpoI**	PmlI	**SmaI**
ApaI	**BstZI**	**NaeI**	PpuMI	**SnaBI**
AscI	**CspI**	**NgoMIV**	PshAI	**SpeI**
BalI	**Csp45I**	**NheI**	Psp5II	SrfI
BbrPI	EagI	**NotI**	PspAI	SwaI
BglII	**Eco52I**	**NruI**	RsrII	**Tth111I**
Bsp120I	Eco72I	PacI	**SacII**	**XhoI**
BsrGI	EcoNI	PaeR7I	**SgfI**	**XmaI**

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

V. pSV-β-Galactosidase Control Vector Restriction Sites (continued)

Table 3. Restriction Enzymes That Cut the pSV-β-Galactosidase Control Vector Six or More Times.

AciI	BsrI	**HaeII**	**MboI**	**PvuII**
AcyI	**BsrSI**	**HaeIII**	**MboII**	**RsaI**
AflIII	Bst71I	HgaI	MnlI	**Sau3AI**
AluI	**BstOI**	**HhaI**	MseI	Sau96I
Alw26I	BstUI	**HinfI**	**MspI**	ScrFI
AspHI	**CfoI**	**HpaII**	**MspA1I**	SfaNI
BanI	**DdeI**	HphI	**NciI**	**TaqI**
BbvI	**DpnI**	**Hsp92I**	**NdeII**	TfiI
BsaOI	DpnII	**Hsp92II**	NlaIII	**Tru9I**
BsaHI	DsaI	MaeI	NlaIV	**XhoII**
BsaJI	Fnu4HI	MaeII	NspI	
Bsp1286I	**FokI**	MaeIII	**PvuI**	

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

VI. References

1. Hall, C.V. *et al*. (1983) Expression and regulation of *Escherichia coli lac*Z gene fusions in mammalian cells. *J. Mol. Appl. Genet*. **2**, 101–9.
2. Rosenthal, N. (1987) Identification of regulatory elements of cloned genes with functional assays. *Methods Enzymol*. **152**, 704–20.
3. Silhavy, T. *et al.* (1984) In: *Experiments with Gene Fusions*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 266.
4. Schenborn, E. and Groskreutz, D. (1999) Reporter gene vectors and assays. *Mol. Biotechnol*. **13**, 29–44.
5. Lim, K. and Chae, C.B. (1989) A simple assay for DNA transfection by incubation of the cells in culture dishes with substrates for β-galactosidase. *Biotechniques* **7**, 576–9.
6. Edlund, T. *et al.* (1985) Cell-specific expression of the rat insulin gene: Evidence for role of two distinct 5´ flanking elements. *Science* **230**, 912–6.
7. *Protocols and Applications Guide*, Online Edition. (2006) Promega Corporation.

VII. Related Products

Product	Size	Cat.#
Beta-Glo® Assay System	10ml	E4720
	100ml	E4740
	10 × 100ml	E4780
β-Galactosidase Enzyme Assay System with Reporter Lysis Buffer	65 assays	E2000
Reporter Lysis Buffer	30ml	E3971

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