Technical Bulletin

pAdVAntage™ Vector

INSTRUCTIONS FOR USE OF PRODUCT E1711.

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I. Description

Co-transfection of mammalian cells with the pAdVAntage™ Vector enhances transient protein expression in a variety of cell types by increasing translation initiation.

Transfection of mammalian cells with an expression vector often results in suboptimal expression of the protein of interest. Double-stranded RNA (dsRNA) generated during transfection is thought to activate the dsRNA-activated inhibitor (DAI), one of several enzymes involved in the host cell's antiviral defense system. DAI phosphorylates the translation initiation factor eIF-2, halting translation and therefore protein production (1, reviewed in reference 2; see Figure 1).

However, inhibition of translation by DAI can be overcome with the adenoviral Virus Associated I RNA (VAI RNA) that is produced by RNA polymerase III following co-transfection with the pAdVAntage™ Vector. The VAI RNA binds to DAI, preventing its activation, thereby allowing translation and protein expression (3,4, reviewed in reference 2; see Figure 1).

The pAdVAntage™ Vector contains base pairs 9,831–11,555 of the adenovirus type 2 genome on a 1,724bp SalI–HindIII fragment that encodes the virus-associated RNA genes, VAI and VAII.

II. Product Components and Storage Conditions

Product	Size	Cat.#
pAdVAntage™ Vector	20µg	E1711

pAdVAntage™ Vector is supplied frozen in TE buffer.

Storage Conditions: Store at –20°C.

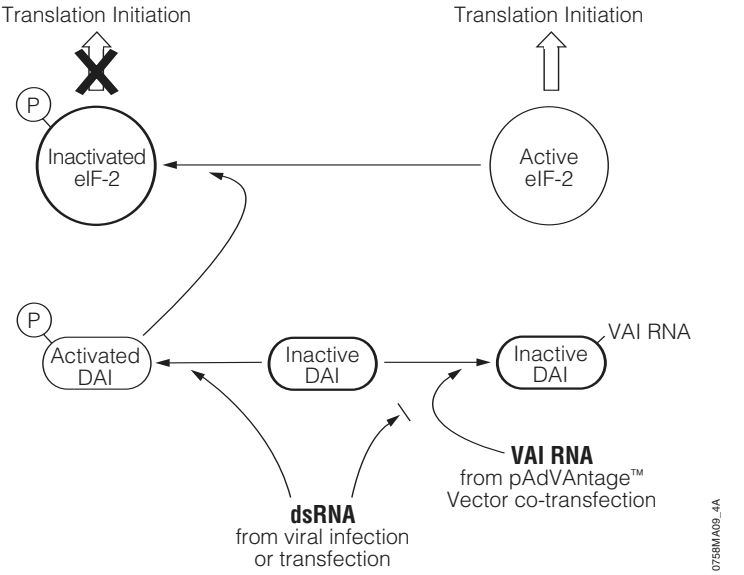

Figure 1. Translation initiation and the effects of VAI RNA on this process.

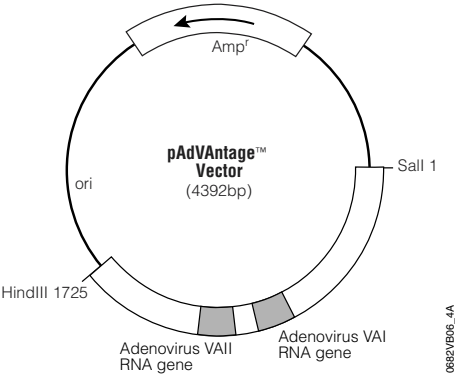

Figure 2. pAdVAntage™ Vector circle map and sequence reference points.

Sequence reference points:

Adenovirus DNA insert	1–1725
Adenovirus VAI RNA gene	780–939
Adenovirus VAII RNA gene	1036–1198
β-lactamase (Amp^r) coding region	2907–3764

III. General Considerations

The level of expression enhancement attained by co-transfection with the pAdVAntage™ Vector is dependent upon a number of parameters, including the cell type, method of transfection and the ratio of pAdVAntage™ Vector DNA to expression vector DNA. Therefore, optimization of these parameters is critical for best results.

III.A. Optimization of the Ratio of pAdVAntage™ Vector to Expression Vector DNA

To optimally enhance protein expression following co-transfection with the pAdVAntage™ Vector, experimentally determine the optimal ratio of pAdVAntage™ Vector to expression vector DNA for each transfection system. For initial optimization experiments, we recommend using ratios of pAdVAntage™ Vector to expression vector DNA in the range of 1:10 to 1:1, as demonstrated in the sample in Table 1.

Table 1. Sample Optimization of the Ratio of Co-Transfected DNAs.

	DNA Ratios (pAdVAntage™ Vector DNA:Expression Vector DNA)			
Transfected DNA	**1:10**	**1:5**	**1:2.5**	**1:1**
pAdVAntage™ Vector DNA (or pBR322 DNA in control plates)	0.5µg	1µg	2µg	5µg
Expression Vector DNA	5µg	5µg	5µg	5µg

III.B. Sample Experiments Demonstrating pAdVAntage™ Vector Enhancement of Expression in HeLa and 293 Cells

To determine how well the pAdVAntage™ Vector can enhance protein expression, we performed co-transfection experiments of 293 and HeLa cells with pGL2-Control DNA and either pAdVAntage™ DNA or pBR322 DNA as a control.

The cells were transfected in 60mm tissue culture dishes with 5µg of the pGL2-Control DNA (Cat.# E1611) using the ProFection® Mammalian Transfection System—Calcium Phosphate (Cat.# E1200). The ratio of pAdVAntage™ DNA or pBR322 DNA co-transfected with the pGL2-Control DNA ranged from 1:10 to 1:1 as detailed in Table 1. The cells were lysed forty-eight hours post-transfection, and luciferase activity expressed from the pGL2-Control DNA was measured using the Luciferase Assay System (Cat.# E1500). The relative-fold increase in luciferase activity due to co-transfection with the pAdVAntage™ Vector was determined using the luciferase activity of the control cells co-transfected with pBR322 as a baseline for comparison.

In these experiments, 293 cells co-transfected with the pAdVAntage™ Vector demonstrated a 10- to 70-fold increase in luciferase expression from pGL2-Control DNA, while HeLa cells demonstrated a 4- to 40-fold increase in luciferase expression when co-transfected with the pAdVAntage™ Vector.

IV. pAdVAntage™ 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 also available from GenBank® database (GenBank®/EMBL Accession Number **U47294**) and on the Internet at: **www.promega.com/vectors/**

Table 1. Restriction Enzymes That Cut the pAdVAntage™ Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location
AatII	2	914, 3899
AccI	2	2, 314
AccIII	1	1171
Acc65I	1	4372
AflIII	2	1656, 2084
Alw44I	3	2398, 3644, 4141
AlwNI	3	494, 1500, 2500
ApaI	1	1377
AvaI	3	1101, 1528, 4376
BalI	2	382, 982
BamHI	2	850, 4381
BbeI	3	207, 1371, 4203
BbrPI	1	1659
BbsI	1	566
BglI	4	265, 726, 3097, 4215
BsaI	1	3038
BsaAI	2	1485, 1659
BsaMI	1	44
BsmI	1	44
Bsp120I	1	1373
BspHI	3	2804, 3812, 3917
BspMI	2	77, 310
BssHII	4	646, 992, 1648, 1650
BssSI	3	2257, 3641, 3948
BstEII	3	407, 878, 1677
BstXI	1	21
BstZI	3	855, 1108, 1664
Cfr10I	5	121, 627, 720, 1106, 3057
CspI	1	1631
Csp45I	1	841
DraI	3	2843, 2862, 3554
DraII	3	1084, 1407, 3953
DrdI	2	2192, 4061
DsaI	5	701, 790, 1325, 1363, 1489
EagI	3	855, 1108, 1664
EarI	5	215, 791, 1968, 3772, 4260
EclHKI	2	321, 2977
Eco47III	1	930
Eco52I	3	855, 1108, 1664
Eco72I	1	1659
EcoICRI	2	425, 4368
EcoNI	1	306
EcoRI	1	4360
EcoRV	2	499, 595
EheI	3	205, 1369, 4201
FseI	1	1110
FspI	5	43, 682, 734, 3199 4222
HincII	3	3, 398, 743
HindII	3	3, 398, 743
HindIII	1	1725
HpaI	1	398
KasI	3	203, 1367, 4199
KpnI	1	4376
NaeI	2	629, 1108
NarI	3	204, 1368, 4200
NcoI	1	701

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

IV. pAdVAntage™ Vector Restriction Sites (continued)

Table 1. Restriction Enzymes That Cut the pAdVAntage™ Vector Between 1 and 5 Times (continued).

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
NdeI	1	4148	**SacI**	2	427, 4370
NgoMIV	2	627, 1106	**SacII**	3	1328, 1366, 1492
NheI	1	969	**SalI**	1	1
NotI	1	1664	**ScaI**	1	3457
NruI	1	1508	**SmaI**	1	4378
NsiI	1	1214	SplI	2	1481, 1685
NspI	3	1280, 2088, 4005	**SspI**	1	3781
PmlI	1	1659	**StyI**	4	20, 701, 1067, 1447
Ppu10I	1	1210			
PpuMI	1	1084	TfiI	2	1919, 2059
Psp5II	1	1084	**Tth111I**	1	468
PspAI	1	4376	**VspI**	3	1855, 1914, 3149
PvuI	2	3347, 4243	**XbaI**	2	749, 4387
PvuII	3	1458, 1908, 4272	**XmaI**	1	4376
RsrII	1	1631	**XmnI**	1	3576

Table 2. Restriction Enzymes That Do Not Cut the pAdVAntage™ Vector.

AccB7I	BlpI	DraIII	PmeI	**SphI**
AflII	Bpu1102I	Eco81I	PshAI	SrfI
AgeI	BsaBI	**I-PpoI**	**PstI**	Sse8387I
AscI	BsrGI	**MluI**	**SfiI**	**StuI**
AvrII	Bst1107I	PacI	**SgfI**	SwaI
BbuI	**Bst98I**	PaeR7I	SgrAI	XcmI
BclI	**Bsu36I**	PflMI	**SnaBI**	**XhoI**
BglII	**ClaI**	PinAI	**SpeI**	

Table 3. Restriction Enzymes That Cut the pAdVAntage™ Vector 6 or More Times.

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

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

V. Related Products

Product	Size	Cat.#
ProFection® Mammalian Transfection System—Calcium Phosphate	40 transfections	E1200
ProFection® Mammalian Transfection System—DEAE-Dextran	40 transfections	E1210
Transfectam® Reagent for the Transfection of Eukaryotic Cells	1mg	E1231
	0.5mg	E1232
TransFast™ Transfection Reagent	1.2mg	E2431
Tfx™-20 Reagent	4.8mg	E2391
Tfx™-50 Reagent	2.1mg	E1811
Tfx™ Reagents Transfection Trio	5.4mg	E2400
pSI Mammalian Expression Vector	20µg	E1721
pCI Mammalian Expression Vector	20µg	E1731
pCI-neo Mammalian Expression Vector	20µg	E1841
pTARGET™ Mamalian Expression Vector	20 reactions	A1410

VI. References

1. Farrell, P.J. *et al.* (1977) Phosphorylation of initiation factor eIF-2 and the control of reticulocyte protein synthesis. *Cell* **11**, 187–200.
2. Groskreutz, D. and Schenborn, E. (1994) Increased gene expression in mammalian cell lines using pAdVAntage™ DNA as a co-transfectant. *Promega Notes* **48**, 8–12.
3. Kitajewski, J. *et al.* (1986) Adenovirus VAI RNA antagonizes the antiviral action of interferon by preventing activation of the interferon-induced eIF-2 alpha kinase. *Cell* **45**, 195–200.
4. O'Malley, R. *et al.* (1986) A mechanism for the control of protein synthesis by adenovirus VA RNAI. *Cell* **44**, 391–400.

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