Antibody Design LABORATORIES

TGEX™-HC-dG3-Zeo Expression Vector

INSTRUCTION MANUAL

TGEX™-HC-dG3-Zeo Transient Mammalian Expression Vector

Catalog #: MX048

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Description

Introduction

The **TGEX™** vector series is designed for the rapid expression of antibody molecules by transient gene expression in mammalian cells in suspension culture. This new series, version 7, also allows the selection of stable transformants through the use of Zeocin®. This vector series features a cytomegalovirus (CMV) promoter, the adenovirus tripartite leader sequence (TPL) (Logan 1984, Mariati 2010), a composite leader sequence (hybrid between a mammalian kappa leader and the bacterial pelB leader (Valadon 2006)) followed by a universal double-inverted BsaI cloning site to insert antibody variable regions. The constant regions are derived from species-specific IgG, kappa and lambda sequences. The 3' end on the transcription unit is composed of an IRES element, the Zeocin-resistance gene (Sh ble from *Streptoalloteichus hindustanus*), a Woodchuck hepatitis virus post-transcriptional regulatory element, and the rabbit beta-globin polyadenylation signal. Transfection of the **TGEX™** expression vectors harboring antibody variable region inserts in widely available cell lines using large-scale transfection technologies (see experimental procedure) typically yields antibody titers between 50 and 250 mg/L in serum-free conditions in just a few days.

The **TGEX™-HC-dG3-Zeo** vector is designed for the expression of a heavy chain variable region with the constant region of the dog IgG1 heavy chain. Expression of full-length antibody molecules is achieved by co-transfection with a light chain variable region cloned into one the **TGEX™** vectors, see below examples to achieve varied antibody formats.

Combination of vectors to desired antibody format (examples)

COMBINATION	FORMAT	PURIFICATION
TGEX™-HC-hG1-Zeo + TGEX™-LC-hK-Zeo	Full length human or chimeric human IgG1/K	Protein A or G
TGEX™-FH-hG1-Zeo + TGEX™-LC-hK-Zeo	Human or chimeric IgG1/K Fab fragment	Protein L, G, or IMAC
TGEX™-FC-hG1-Zeo	Human IgG1 Fc fusion	Protein A or G
TGEX™-SCblue-Zeo	Human IgG1 scFv-Fc fusion	Protein A or G

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
TGEX™-HC-dG3-Zeo	20 µl at 0.5 µg/µl of DNA vector in DNA Conservation Buffer (Tris-HCL 5 mM, EDTA 0.1 mM, pH 8.5)	10 µg

Shipping & Storage

TGEX™-HC-dG3-Zeo vector is shipped on wet ice. Upon receipt, store the vector at -20°C.

Limited Product Warranty

This warranty limits our liability to the replacement of this product. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Antibody Design Labs. Antibody Design Labs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

For research use only; not intended for any animal or human therapeutic or diagnostic use.

TGEX™ Vector Series

TGEX™ vector series for desired isotypes

VECTOR	CATALOG	ISOTYPE	DESCRIPTION
TGEX™-HC-hG1-Zeo	MX026	Human IgG1	Heavy chain expression plasmid for human IgG1
TGEX™-FH-hG1-Zeo	MX023	Human IgG1 CH1	For the expression of human IgG1 Fab fragments
TGEX™-FC-hG1-Zeo	MX025	Human IgG1 Fc	For the expression of human IgG1 Fc fusions
TGEX™-HC-hG2-Zeo	MX027	Human IgG2	Heavy chain expression plasmid for human IgG2
TGEX™-HC-hG3-Zeo	MX028	Human IgG3	Heavy chain expression plasmid for human IgG3
TGEX™-HC-hG4[S228P]-Zeo	MX029	Human IgG4[S228P]	Heavy chain expression plasmid for human IgG4
TGEX™-LC-hK-Zeo	MX030	Human Kappa	Light chain expression plasmid for human Kappa
TGEX™-LC-hL2-Zeo	MX031	Human Lambda 2	Light chain expression plasmid for human Lambda 2
TGEX™-HC-mG1-Zeo	MX032	Murine IgG1	Heavy chain expression plasmid for murine IgG1
TGEX™-HC-mG2a-Zeo	MX033	Murine IgG2a	Heavy chain expression plasmid for murine IgG2a
TGEX™-HC-mG2b-Zeo	MX034	Murine IgG2b	Heavy chain expression plasmid for murine IgG2b
TGEX™-HC-mG3-Zeo	MX035	Murine IgG3	Heavy chain expression plasmid for murine IgG3
TGEX™-LC-mK-Zeo	MX036	Murine Kappa	Light chain expression plasmid for murine Kappa
TGEX™-LC-mL1-Zeo	MX037	Murine Lambda 1	Light chain expression plasmid for murine Lambda 1
TGEX™-LC-mL2-Zeo	MX038	Murine Lambda 2	Light chain expression plasmid for murine Lambda 2
TGEX™-HC-rbG-Zeo	MX039	Rabbit IgG	Heavy chain expression plasmid for rabbit IgG
TGEX™-LC-rbKb4-Zeo	MX040	Rabbit Kappa	Light chain expression plasmid for rabbit Kappa
TGEX™-HC-dG1-Zeo	MX046	Dog IgG1	Heavy chain expression plasmid for dog IgG1
TGEX™-HC-dG2-Zeo	MX047	Dog IgG2	Heavy chain expression plasmid for dog IgG2
TGEX™-HC-dG3-Zeo	MX048	Dog IgG3	Heavy chain expression plasmid for dog IgG3
TGEX™-HC-dG4-Zeo	MX049	Dog IgG4	Heavy chain expression plasmid for dog IgG4
TGEX™-LC-dK-Zeo	MX050	Dog Kappa	Light chain expression plasmid for dog Kappa
TGEX™-LC-dL-Zeo	MX051	Dog Lambda	Light chain expression plasmid for dog Lambda

TGEX™ vector series for Fc-engineered antibodies

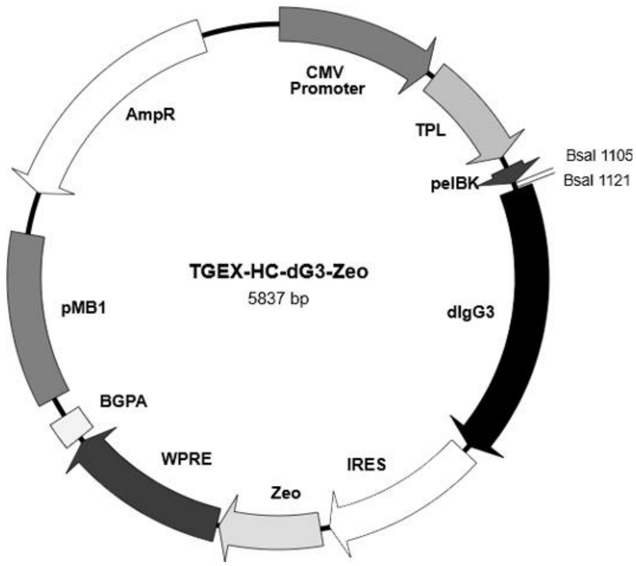
VECTOR	CATALOG	ISOTYPE	DESCRIPTION
TGEX™-HC-hG1[EA]-Zeo	MX041	Human IgG1	Human IgG1 heavy chain with increased ADCC/CDC *in vitro*
TGEX™-HC-hG1[NA]-Zeo	MX042	Human IgG1	Human IgG1 aglycosylated heavy chain
TGEX™-HC-hG1[LALA-PG]-Zeo	MX043	Human IgG1	Human IgG1 heavy chain with decreased ADCC/CDC *in vitro*
TGEX™-HC-hG1[YTE-KF]-Zeo	MX044	Human IgG1	Human IgG1 heavy chain with increased serum half-life
TGEX™-HC-hG4[SPLE-PG]-Zeo	MX045	Human IgG4[S228P]	Human IgG4 heavy chain with decreased ADCC/CDC *in vitro*

TGEX™ control vector, universal expression and Fc fusions

VECTOR	CATALOG	USE	DESCRIPTION
TGEX™-AC-Zeo	MX020	Any expressions	Universal expression vector
TGEX™-eGFP-Zeo	MX022	Transfection	Control plasmid for monitoring transient transfections
TGEX™-SCblue-Zeo	MX024	scFv cloning vector	For the transfer of scFv from any PADL phagemid vector and expression as an scFv-Fc fusion

Vector Map

The figure below illustrates the main features of **TGEX™-HC-dG3-Zeo** expression vector. The full vector sequence is available online for download in varied formats on the product web page; the total length of the vector is 5828 bp.



Cloning Site

Following is an illustration of **TGEX™-HC-dG3-Zeo** cloning site from the EcoRI site and onward. The VH domain is inserted in a double inverted BsaI cloning site located between the pelBK leader sequence and the constant regions. The four base pair overhangs after a restriction digestion with BsaI are boxed and grayed.

```
       tgex-S3                                                       EcoRI
       ------------------->                                              |
 971   GAAAGGCGTC TAACCAGTCA CAGTCGCAAG TTTAAACGGA TCTCTAGCGA
                             pelBK leader sequence
                             MetGLuThrA spThrLeuLe uLeuTrpVal
1021   ATTCGGCTTG GGCCGCCACC ATGGAGACAG ACACACTCCT GCTATGGGTA

                   SfiI                        <BsaI        MluI
                     |                           |            |
       LeuLeuLeuL euAlaAlaGl nProAlaMet Ala
1071   CTGCTGCTCT TAGCGGCCCA GCCGGCCATG GCAGGAGACC TAACGCGTAT

       >BsaI      dIgG3
       |          ┌------------------------------------------...
                 Ala SerThrThrA laProSerVa lPheProLeu AlaProS...
1121   GGTCTCAGCC TCCACCACGG CCCCCTCGGT TTTCCCACTG GCCCCCAGCT
```

Feature Table

The features of **TGEX™-HC-dG3-Zeo** transient expression vector are highlighted in the following table.

FEATURE	LOCATION	DESCRIPTION
Promoter	5-585	CMV promoter.
TPL	612-1000	Adenovirus tripartite leader sequence (Logan 1984, Mariati 2010).
pelBK leader	1041-1103	Hybrid kappa/pelB leader peptide sequence. The cleavage occurs on the C-terminal side of the terminal alanine.
Dog IgG3 CDS	1128-2129	Sequence encoding the dog IgG3 heavy chain sequence comprising the CH1, CH2, and CH3 domains with the hinge region. The sequence is intronless; the C-terminal lysine residue is encoded.
IRES	2155-2728	Internal Ribosome Entry Site.
Zeo	2762-3137	Sh ble gene from *Streptoalloteichus hindustanus* conferring resistance to Zeocin.
WPRE	3145-3733	Woodchuck hepatitis virus post-transcriptional regulatory element.
BGpA	3749-3847	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	3921-4540	pBR322 origin for replication in *E. coli* with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	5555- 4695	Ampicillin resistance for selection in *E. coli*.

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AlfI	(10/12)GCANNNNNNTGC(12/10)	1	3099		
AlwNI	CAGNNN^CTG	1	4286		CaiI PstNI
ApaI	GGGCC^C	1	2268		Bsp120I PspOMI
ArsI	(8/13)GACNNNNNNTTYG(11/6)	1	851		
AvrII	C^CTAGG	1	2306		AspA2I BlnI XmaJI
BamHI	G^GATCC	1	2744		
BbvCI	CCTCAGC(-5/-2)	1	1319		
BcgI	(10/12)CGANNNNNNTGC(12/10)	1	5284		
BplI	(8/13)GAGNNNNNCTC(13/8)	1	1779		
BsaXI	(9/12)ACNNNNNCTCC(10/7)	1	2998	-	
BsePI	G^CGCGC	1	2798		BssHII PauI PteI
BsgI	GTGCAG(16/14)	1	1596		
Bsp1407I	T^GTACA	1	5799		BsrGI BstAUI
BstEII	G^GTNACC	1	1865		BstPI Eco91I EcoO65I PspEI
CspCI	(11/13)CAANNNNNGTGG(12/10)	1	407		
DrdI	GACNNNN^NNGTC	1	3977		AasI DseDI
Eam1105I	GACNNN^NNGTC	1	4763		AhdI BmeRI DriI
EcoNI	CCTNN^NNNAGG	1	1342		BstENI XagI
EcoRV	GAT^ATC	1	2099		Eco32I
Esp3I	CGTCTC(1/5)	1	1256		BsmBI
FalI	(8/13)AAGNNNNNCTT(13/8)	1	784		
FseI	GGCCGG^CC	1	3033		RigI
FspI	TGC^GCA	1	4988		Acc16I NsbI
KflI	GG^GWCCC	1	1173		
KpnI	GGTAC^C	1	2596		Acc65I Asp718I
MauBI	CG^CGCGCG	1	2797		
MluI	A^CGCGT	1	1113		
NheI	G^CTAGC	1	2133		AsuNHI BmtI BspOI
NotI	GC^GGCCGC	1	3137		CciNI
NsiI	ATGCA^T	1	2067		EcoT22I Mph1103I Zsp2I
OliI	CACNN^NNGTG	1	1556		AleI
PasI	CC^CWGGG	1	1796		
PciI	A^CATGT	1	2645		PscI

PmaCI	CAC^GTG	1	2469		AcvI BbrPI Eco72I PmlI PspCI
PmeI	GTTT^AAAC	1	1000		MssI
PstI	CTGCA^G	1	1296		BspMAI
PvuI	CGAT^CG	1	5135		Ple19I
SacI	GAGCT^C	1	583		Ecl136II EcoICRI Eco53kI Psp124BI SstI
SalI	G^TCGAC	1	2756		
SexAI	A^CCWGGT	1	2925		CsiI MabI
SgrAI	CR^CCGGYG	1	2875		
SnaBI	TAC^GTA	1	357		BstSNI Eco105I
SpeI	A^CTAGT	1	18		AhlI BcuI
XbaI	T^CTAGA	1	2750		
XcmI	CCANNNNN^NNNNTGG	1	1189		
XhoI	C^TCGAG	1	966		Sfr274I PaeR7I SlaI
BglII	A^GATCT	2	3864		
		2	5688		
Bpu10I	CCTNAGC(-5/-2)	2	930		
		2	1319		
BspHI	T^CATGA	2	4595		CciI PagI
		2	5603		
Bsu36I	CC^TNAGG	2	1303		Eco81I AxyI Bse21I
		2	1590		
BtrI	CACGTC(-3/-3)	2	2696	-	AjiI BmgBI
		2	2894		
BtsI	GCAGTG(2/0)	2	5161		
		2	5189		
DraIII	CACNNN^GTG	2	2513		AdeI
		2	3110		
EagI	C^GGCCG	2	3093		BseX3I BstZI EclXI Eco52I
		2	3138		
EcoRI	G^AATTC	2	1019		
		2	1250		
GsuI	CTGGAG(16/14)	2	1343	-	BpmI
		2	4853		
HindIII	A^AGCTT	2	2727		
		2	3870		
SacII	CCGC^GG	2	740		Sfr303I KspI SgrBI Cfr42I
		2	3645		
SfiI	GGCCNNNN^NGGCC	2	1085		
		2	1382		
SmaI	CCC^GGG	2	2740		Cfr9I TspMI XmaI
		2	2850		
VspI	AT^TAAT	2	25		AseI PshBI
		2	4939		

Absent Sites:
AanI, AbsI, AccIII, AfeI, AflII, AgeI, AjuI, AloI, Aor13HI, Aor51HI, AscI, AsiGI, AsiSI, AsuII, BaeI, BarI, BclI, BfrI, BlpI, BoxI, Bpu1102I, Bpu14I, Bsa29I, BsaBI, Bse8I, BseAI, BseCI, BseJI, BshTI, BshVI, BsiWI, Bsp119I, Bsp13I, Bsp1720I, Bsp68I, BspDI, BspEI, BspQI, BspT104I, BspTI, BssNAI, Bst1107I, BstAFI, BstAPI, BstBI, BstPAI, BstXI, BstZ17I, Bsu15I, BsuTUI, BtuMI, ClaI, CpoI, CspAI, CspI, DinI, Eco147I, Eco47III, EgeI, EheI, FbaI, FspAI, HpaI, I-CeuI, I-PpoI, I-SceI, KasI, Kpn2I, Ksp22I, KspAI, LguI, MfeI, Mly113I, MreI, MroI, MspCI, MunI, NarI, NruI, NspV, PI-PspI, PI-SceI, PacI, PaeI, PalAI, PceI, PciSI, Pfl23II, PflFI, PinAI, PluTI, PshAI, PsiI, PspLI, PspXI, PsrI, PsyI, RgaI, RruI, Rsr2I, RsrII, SapI, SbfI, SdaI, SfaAI, SfoI, SfuI, SgfI, SgrDI, SgsI, SmiI, SphI, SrfI, Sse8387I, SseBI, SspDI, StuI, SwaI, Tth111I, Vha464I.

Experimental Procedures

General Molecular Biology Techniques

Molecular biology should be conducted under the supervision of a qualified instructor trained to standard safety practice in a molecular biology laboratory environment. Standard molecular biology procedures can be found in a general molecular biology handbook such as Sambrook (1989).

Plasmid Maintenance

Propagation and maintenance of TEGX™ vectors is obtained on any *recA1, endA1 E. coli* strain using LB or 2xYT medium supplemented with ampicillin (100 µg/ml) as a selection marker and incubated at 37°C with agitation. TEGX™ vectors are derivatives of pBR322 with a high copy number origin of replication and usually gives high yields of plasmid DNA with most standard laboratory strains such as XL1-blue or DH5α. The high copy number phenotype is temperature-sensitive and requires incubation at 37°C (Lin-Chao 1992). Some DNA stabilizing strains are known to produce smaller amounts of plasmid DNA. In case of issues, we recommend using XL10-Gold® from Agilent Technologies, Inc., on which TGEX™ plasmid DNA can be isolated in large quantities.

Cloning into TGEX™-HC-dG3-Zeo

In Silico Design

A double inverted BsaI cloning site separates the pelBK leader peptide sequence from the beginning of the antibody constant region. After digestion with BsaI, the heavy chain variable domain is inserted in-frame between the two elements as illustrated below. During the cloning, the two BsaI sites are eliminated.

```
                          SfiI                                          dIgG3
                           |                                            ┌──────────────────────────────
      LeuLeuLeuL euAlaAlaGl nProAlaMet Ala                             Ala SerThrThrA laProSerVa
1071  CTGCTGCTCT TAGCGGCCCA GCCGGCCATG GCA    [VH]     GCC TCCACCACGG CCCCCTCGGT
```

Vector Digestion

BsaI alone is sufficient to open the cloning site. Please, consult the documentation of your restriction enzyme provider for optimal conditions.

Cloning with FAST-Licase™

Homologous recombination is the easiest and most efficient method to clone seamlessly antibodies into TGEX™ vectors. We recommend the **FAST-Licase™** (Antibody Design Labs cat# MB101S & MB101L) with the following overhangs. For synthetic dsDNA constructs, the overhangs are added on each side. For PCR-amplified constructs, primers should contain the overhang followed by the antibody priming area. The **FAST-Licase™** reaction contains the insert plus the purified vector digested with BsaI (see kit instructions).

pelBK overhang 5′– CGGCCCAGCCGGCCATGGCA

dIgG3 overhang 5′– GCCTCCACCACGGCCCCCTC (reverse complement for PCR primers)

Primer Design for Restriction Cloning with the BsaI Sites

Oligo1 is an example of primer designed to amplify a VH domain sequence and clone it into the BsaI site situated next to the pelBK leader. A minimum of 2 nucleotides is recommended to cut BsaI site close to the end (source New England BioLabs); these 2 nucleotides are followed by the BsaI site GGTCTC and the last five nucleotides of the leader sequence; the resulting NNGGTCTCTGGCA extension is added 5' to the VH domain primer.

Oligo1 5'-NNGGTCTCTGGCA-VH-start

Oligo2 is an example of primer designed to amplify the VH domain from the end of the J region including a BsaI site compatible with **TGEX™-HC-dG3-Zeo** cloning site.

BsaI is a type IIS restriction enzyme that cuts outside of its recognition site. The second BsaI site of the cloning site will be cut immediately before the start of the human IgG1 constant region sequence, exactly 1 base after the end of the site and 5 bases further on the opposite strand, thus freeing a 5' 4-base overhang GCCT on the sense strand. In Oligo2, a BsaI recognition site is situated symmetrically to the vector and will generate a complementary overhang.

Oligo2 5'-NNGGTCTCGAGGC-JH-end

After digestion and ligation, all the two BsaI sites, from both insert and vector, will be removed, resulting in a scarless insertion of the antibody domain.

Alternative to BsaI Sites

All restriction enzymes that generate 4-base long 5' overhangs can be used in place of BsaI; this is the case for example of BsmBI (CGTCTC(1/5)) , another type IIS restriction enzyme. This option could come in handy when the VH domain contains another BsaI preventing cloning.

Sequencing of Inserts

The following primers give a strong PCR amplification of the TGEX™ vector series inserts and the antibody constant regions between the EcoRI site and the NheI site. The primer tgex-S3 can be used to sequence the VH domain in full.

tgex-S3 5'- AGGCGTCTAACCAGTCACAGTC

ires-R 5'- GAATAAGGCCGGTGTGCGTT

Antibody Expression

Cell Lines

Cell lines adapted for culture in suspension and serum-free conditions are recommended. HEK293 and CHO cells are often used for antibody expression by transient transfection; you can either adapt your own cell line or obtain it from a supplier (e.g. Life Technology). HEK293 cells are particularly well suited for expression using **TGEX™** vector series.

Transient Transfection

Many transfection reagents especially designed for transient transfection are commercially available from different providers (e.g. Life Technologies, Mirus Bio LLC, etc.). We recommend testing the transfection conditions with a reporter plasmid first to determine the percentage of cells effectively transfected and optimal transfection conditions; fluorescent reporters are often used with that purpose, e.g. TGEX**™**-eGFP-Zeo (Antibody Design Labs cat# MX022). Similarly, any condition known to boost expression should be carefully tested in your system before being scaled up. We did observe an increase in expression in HEK293 cells upon exposure to sodium valproate (Backliwal 2008). Boosters and enhancers are often included in commercially available transfection kits.

Stable Cell Line Selection

Zeocin can be used very effectively to select stable cell lines secreting antibodies in a few weeks. The following protocol are suggestions that require adjustment to your particular constructs:

WEEK 1.

- Day 0: Transfect in duplicate cells in a 6-well plate containing 2 ml culture per well;
- Day 3-4: Expand each well in two wells with 2 ml culture per well.
- Day 6: Increase the volume to 4 ml per well and add Zeocin at 100 µg/ml.

WEEK 2-3

- Maintain a good cell density and the Zeocin concentration while replenishing the culture with fresh medium as needed.

WEEK 3-4

- Continue selection with possibly a higher Zeocin concentration (up to 1000 µg/ml).
- Proceed to single cloning and analyze stable transformants for expression.

Light chain to Heavy chain ratio

We recommend starting with a 1:1 light chain to heavy chain ratio during transfection. We observed many antibodies with a better expression at a 2:1 light chain to heavy chain ratio although each antibody requires fine tuning for optimal expression.

Appendix

MSDS Information

Material Safety Data Sheets are available on Antibody Design Labs website at the corresponding product page.

Quality Control

Specifications and quality control are detailed on the online product page. Antibody Design Labs certifies that the product will perform according to these specifications.

Technical Support

Visit Antibody Design Labs website at **www.abdesignlabs.com** for technical resources, including manuals, vector maps and sequences, application notes, FAQs, etc.

For more information or technical assistance, call, write, or email us at:

Antibody Design Labs
4901 Morena Blvd, Suite 203
San Diego, CA 92117

Email: support@abdesignlabs.com
Phone: 1-877-223-3104 (Toll-Free)
(Monday – Friday 9:00 AM – 5:00 PM PST)

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