TGEX-SCBlue-Zeo Expression Vector

INSTRUCTION MANUAL

TGEX-SCBlue-Zeo Mammalian Expression Vector

Catalog #: MX024

Version: A1.2 – February 2024

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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™-SCblue-Zeo** vector is designed for the transient mammalian expression of scFv-Fc fusions after transfer of scFv fragments from the **PADL™** phagemid vector series. **TGEX™-SCblue-Zeo** vector enables rapid and convenient expression of scFv fragment isolated by phage display as dimeric scFv-Fc fusion with all the advantages conferred by the Fc fragment for detection using commercially available secondary antibodies. The pelBK signal peptide responsible for the secretion of the Fc fusion in the culture supernatant is a composite between a mammalian kappa leader sequence and the bacterial pelB leader sequence with a compatible SfiI restriction site for the transfer (Valadon 2006). Proper recombinant clones can be isolated using a blue/white colony screening.

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
TGEX-SCBlue-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-SCBlue-Zeo vector is shipped on wet ice. Upon receipt, store the vector at -20°C.

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

TGEX™ Vector Series

TGEX™ vectors 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™ vectors for Fc-engineered antibodies

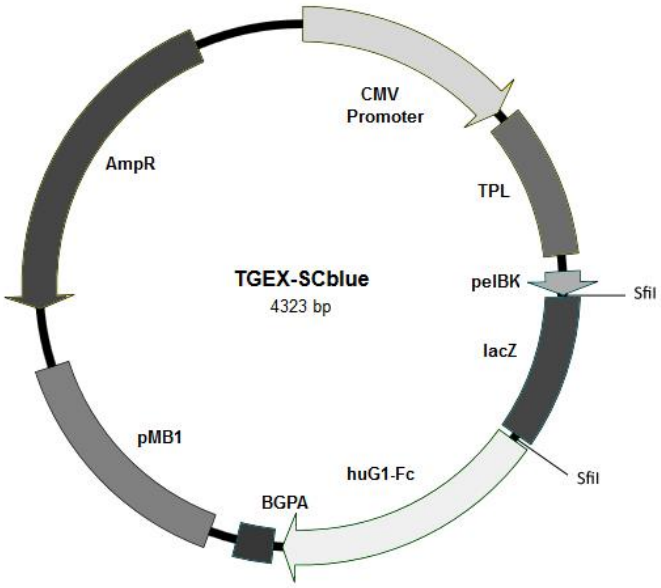
VECTOR	CATALOG	ISOTYPE	DESCRIPTION
TGEX™-HC-hG1[EA]-Zeo	MX041	Human IgG1	Human IgG1 heavy chain with increased ADCC/CDC *in vitro*
TGEX™-HC-hG1[NA]-Zeo	MX042	Human IgG1	Human IgG1 aglycosylated heavy chain
TGEX™-HC-hG1[LALA-PG]-Zeo	MX043	Human IgG1	Human IgG1 heavy chain with decreased ADCC/CDC *in vitro*
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-SCBlue-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 5917 bp.



Cloning Site

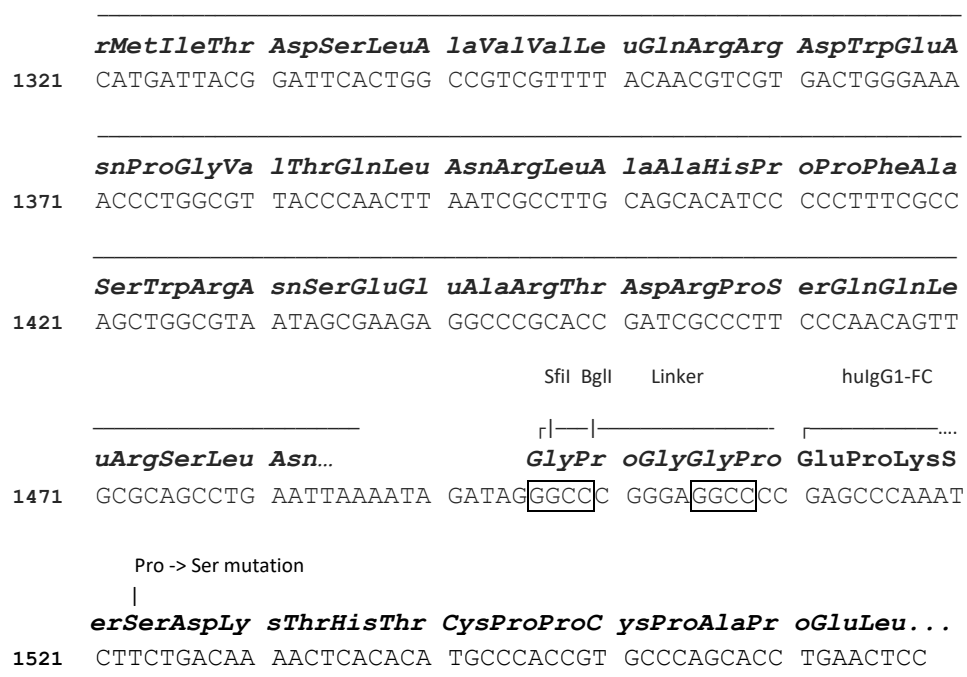
Following is an illustration of **TGEX-SCBlue-Zeo** cloning site from the EcoRI site and onward. The scFv is inserted in a double inverted BsaI cloning site located between the pelBK leader sequence and the constant regions. The four base pair overhangs after a restriction digestion with BsaI are boxed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

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

                      SfiI BglI                    lacZ
                        |  |                       ┌─────---
      LeuLeuLeuL euAlaAlaGl nProAlaMet Ala
1071  CTGCTGCTCT TAGCGGCCCA GCCGGCCATG GCGCCCAATA CGCAAACCGC

1121  CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT
1171  CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTAGCT
1221  CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT

                                                    Alpha-B-gal
                                                    ┌──────
                                                    MetTh
1271  TGTGTGGAAT TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC
```



Feature Table

The features of **TGEX-SCBlue-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.
lacZ	1104-1495	LacZ fragment with lac promoter.
Alpha-B-gal	1316-1486	B-gal alpha fragment.
Human IgG1-FC CDR	1511-2210	Sequence encoding the human IgG1 FC sequence comprising the CH2, and CH3 domains with the hinge region. The sequence is intronless; the C-terminal lysine residue is encoded.
IRES	2235-2808	Internal Ribosome Entry Site.
Zeo	2842- 3216	Sh ble gene from *Streptoalloteichus hindustanus* conferring resistance to Zeocin.
WPRE	3225-3813	Woodchuck hepatitis virus post-transcriptional regulatory element.
BGpA	3829-3927	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	4001 -4620	pBR322 origin for replication in *E. coli* with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	5635-4775	Ampicillin resistance for selection in *E. coli*.

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AlfI	(10/12)GCANNNNNNTGC(12/10)	1	3179		
ArsI	(8/13)GACNNNNNNTTYG(11/6)	1	851		
AvrII	C^CTAGG	1	2386		AspA2I BlnI XmaJI
BamHI	G^GATCC	1	2824		
BcgI	(10/12)CGANNNNNNTGC(12/10)	1	5364		
Bpu10I	CCTNAGC(-5/-2)	1	930		
BsePI	G^CGCGC	1	2878		BssHII PauI PteI
CspCI	(11/13)CAANNNNNGTGG(12/10)	1	407		
EcoNI	CCTNN^NNNAGG	1	1789		BstENI XagI
EcoRI	G^AATTC	1	1019		
FalI	(8/13)AAGNNNNNCTT(13/8)	1	784		
FseI	GGCCGG^CC	1	3113		RigI
GsuI	CTGGAG(16/14)	1	4933	-	BpmI
KpnI	GGTAC^C	1	2676		Acc65I Asp718I
MauBI	CG^CGCGCG	1	2877		
NarI	GG^CGCC	1	1100		DinI EgeI EheI KasI Mly113I PluTI SfoI SspDI
NheI	G^CTAGC	1	2213		AsuNHI BmtI BspOI
NotI	GC^GGCCGC	1	3217		CciNI
NsiI	ATGCA^T	1	2147		EcoT22I Mph1103I Zsp2I
OliI	CACNN^NNGTG	1	1642		AleI
PciI	A^CATGT	1	2725		PscI
PmaCI	CAC^GTG	1	2549		AcvI BbrPI Eco72I PmlI PspCI
PmeI	GTTT^AAAC	1	1000		MssI
SacI	GAGCT^C	1	583		Ecl136II EcoICRI Eco53kI Psp124BI SstI
SalI	G^TCGAC	1	2836		
SapI	GCTCTTC(1/4)	1	2179	-	BspQI LguI PciSI
SgrAI	CR^CCGGYG	1	2955		
SnaBI	TAC^GTA	1	357		BstSNI Eco105I
SpeI	A^CTAGT	1	18		AhlI BcuI
XbaI	T^CTAGA	1	2830		
XhoI	C^TCGAG	1	966		Sfr274I PaeR7I SlaI
AarI	CACCTGC(4/8)	2	2109	-	PaqCI
		2	2564		
AlwNI	CAGNNN^CTG	2	1554		CaiI PstNI
		2	4366		
ApaI	GGGCC^C	2	1495		Bsp120I PspOMI
		2	2348		
BalI	TGG^CCA	2	2843		MlsI MluNI Mox20I MscI Msp20I
		2	5845		
BglII	A^GATCT	2	3944		
		2	5768		
BsgI	GTGCAG(16/14)	2	1790	-	
		2	2159		
BsmI	GAATGC(1/-1)	2	2381	-	Mva1269I PctI
		2	2414		
Bsp1407I	T^GTACA	2	1908		BsrGI BstAUI
		2	5879		
BssSI	CACGAG(-5/-1)	2	4128	-	BauI Bst2BI
		2	5512		
Bsu36I	CC^TNAGG	2	1634		Eco81I AxyI Bse21I
		2	1676		
DraIII	CACNNN^GTG	2	2593		AdeI

		2	3190	
DrdI	GACNNNN^NNGTC	2	1673	AasI DseDI
		2	4057	
EagI	C^GGCCG	2	3173	BseX3I BstZI EclXI Eco52I
		2	3218	
Eam1105I	GACNNN^NNGTC	2	1575	AhdI BmeRI DriI
		2	4843	
FspI	TGC^GCA	2	1470	Acc16I NsbI
		2	5068	
HindIII	A^AGCTT	2	2807	
		2	3950	
NdeI	CA^TATG	2	252	FauNDI
		2	3906	
PvuI	CGAT^CG	2	1450	Ple19I
		2	5215	
SexAI	A^CCWGGT	2	1947	CsiI MabI
		2	3005	
SfiI	GGCCNNNN^NGGCC	2	1085	
		2	1496	

Absent Sites:
AanI, AbsI, AccIII, AfeI, AflII, AgeI, AjuI, AloI, Aor13HI, Aor51HI, AscI,
AsiGI, AsiSI, AsuII, BaeI, BarI, BbvCI, BclI, BfrI, BlpI, BoxI, BplI, Bpu1102I,
Bpu14I, Bsa29I, BsaBI, BsaI, Bse8I, BseAI, BseCI, BseJI, BshTI, BshVI, BsiWI,
BsmBI, Bso31I, Bsp119I, Bsp13I, Bsp1720I, Bsp68I, BspDI, BspEI, BspMAI,
BspT104I, BspTI, BspTNI, BssNAI, Bst1107I, BstAFI, BstAPI, BstBI, BstEII,
BstPAI, BstPI, BstXI, BstZ17I, Bsu15I, BsuTUI, BtuMI, ClaI, CpoI, CspAI, CspI,
Eco147I, Eco31I, Eco32I, Eco47III, Eco91I, EcoO65I, EcoRV, Esp3I, FbaI, FspAI,
HpaI, I-CeuI, I-PpoI, I-SceI, KflI, Kpn2I, Ksp22I, KspAI, MfeI, MluI, MreI,
MroI, MspCI, MunI, NruI, NspV, PI-PspI, PI-SceI, PacI, PaeI, PalAI, PasI, PceI,
Pfl23II, PflFI, PinAI, PshAI, PsiI, PspEI, PspLI, PspXI, PsrI, PstI, PsyI, RgaI,
RruI, Rsr2I, RsrII, SbfI, SdaI, SfaAI, SfuI, SgfI, SgrDI, SgsI, SmiI, SphI,
SrfI, Sse8387I, SseBI, StuI, SwaI, Tth111I, Vha464I, XcmI.

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-SCBlue-Zeo

Primer Design and pelBK Leader Sequence

A complete hybrid leader sequence is necessary for secretion and proper removal of the leader peptide by host proteases. In the following schema, where [NNN] represents the insert sequence and [Xxx] the translated amino acid sequence, the short hexanucleotide ATGGCN must be appended immediately to the first *SfiI* site to obtain a complete hybrid leader encoding sequence.

Transfer from PADL Phagemid Vectors

scFv fragments and VHH domains can be excised from PADL™ phagemids by SfiI or alternatively by BglI and cloned directly into TGEX-SCblue opened by SfiI. Classical blue/white screen can be applied to detect insert-containing clones; IPTG is dispensable thanks to the very high copy number of TGEX-SCblue.

Sequencing of Inserts

The following primers give a strong PCR amplification of the TGEX vector series inserts between the EcoRI site and the NotI site. The primer tgex-S3 can be used to sequence the scFv fragment.

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). 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.

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:

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Phone: 1-877-223-3104 (Toll Free)
(Monday – Friday 9:00 AM – 5:00 PM PST)

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