Antibody Design LABORATORIES

TGEX-FC Expression Vector

INSTRUCTION MANUAL

TGEX-FC Transient Mammalian Expression Vector

Catalog #: MX003

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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 cell suspension culture. The vector series features a CMV promoter, the adenovirus tripartite leader sequence (TPL) (Logan 1984, Mariati 2010), a variable antibody domain leader sequence with its intron and convenient cloning sites to insert antibody variable regions. The constant regions are derived from the human IgG1 and kappa sequences. There is no selection marker and the overall small size of the vectors is optimized for transient transfection; expensive antibiotics to prepare large quantities of plasmid for transient transfections are not required. Using widely available cell lines and large scale transfection technologies (see experimental procedure) yields of antibody between 10 mg/L and 100 mg/L in serum-free conditions are routinely achieved in the laboratory in just a few days.

The **TGEX™-FC** vector is designed for the expression of N-terminal fusions with the Fc region of the human IgG1.

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
TGEX-FC	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-FC vector is shipped on wet ice. Upon receipt, store the vector at -20ºC.

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TGEX Vector Series

Characteristics of the TGEX vector series

VECTOR	CATALOG	USE	DESCRIPTION
TGEX™-HC	MX001	Cloning of VH domain	For the expression of human IgG1 heavy chain
TGEX™-LC	MX002	Cloning of VL domain	For the expression of human Kappa light chain
TGEX™-FC	MX003	Cloning of VH domain	For the expression of human IgG1 Fc fusion.
TGEX™-FH	MX004	Cloning of VH domain	For the expression of human IgG1 Fab fragment
TGEX™-HChis	MX005	Cloning of VH domain	For the expression of human IgG1 heavy chain with a HIS tag
TGEX™-SCblue	MX006	scFv cloning vector	For the transfer of scFv from any PADL phagemid vector and expression as an scFv-Fc fusion

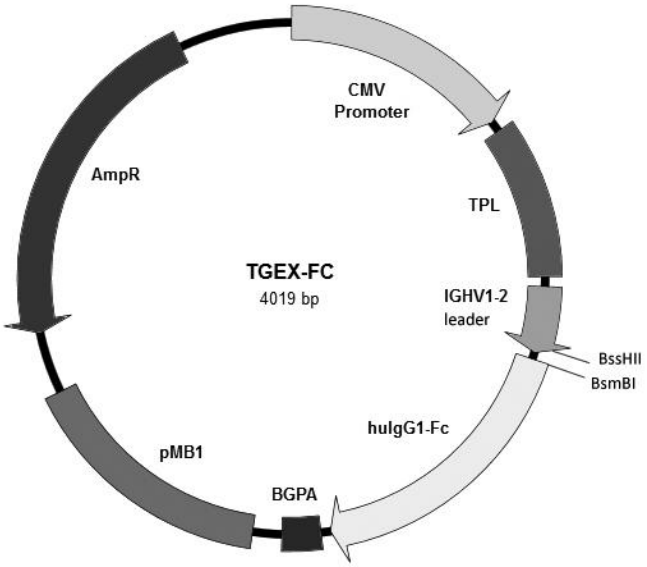
Combination of vectors to desired antibody format

COMBINATION	FORMAT	PURIFICATION[1]
TGEX™-HC + TGEX™-LC	Full length human or chimeric human IgG1/K	Protein A or G
TGEX™-HChis + TGEX™-LC	Full length human or chimeric human IgG1/K	Protein A , G or IMAC
TGEX™-FH + TGEX™-LC	Human or chimeric IgG1/K Fab fragment	Protein L, G or IMAC
TGEX™-FC	Fc fusion	Protein A or G
TGEX™-SCblue	scFv-Fc fusion	Protein A or G

1. Purification by protein A, G and L may require testing.

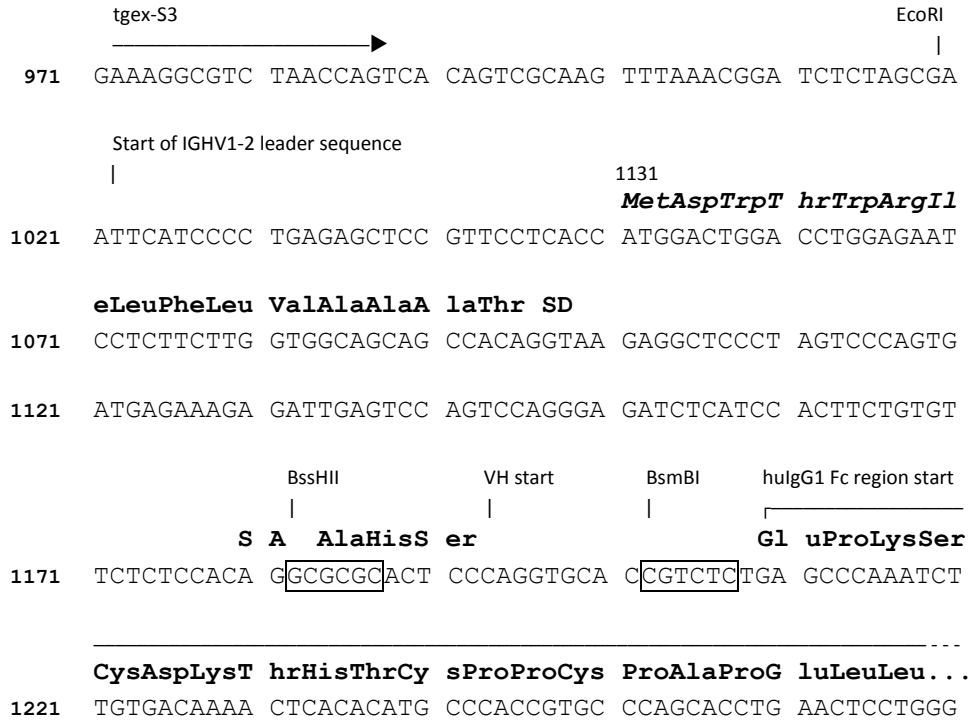
Vector Map

The figure below illustrates the main features of **TGEX-FC** 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 4019 bp.



Cloning Site

Following is an illustration of **TGEX-FC** cloning site from the EcoRI site and onward. The VH domain is inserted between the BSSHII site and the BsmBI site; after excision of the intron sequence, the IGHV1-2 sequence encodes the leader peptide MDWTWRILFLVAAATGAHS. SA/SD donor and acceptor sites.



Feature Table

The features of **TGEX-HC** 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)
IGHV1-2 leader	1025-1192	Human IGHV1-2 leader sequence with intron. The mature RNA encodes the 18 a.a.–long signal sequence MDWTWRILFLVAAATGAHS; cleavage occurs on the C-terminal side of the terminal serine.
Human IgG1 Fc	1209-1907	Sequence encoding the human IgG1 Fc constant region including CH2 and CH3 domains with the hinge region. The sequence is intronless; the proximal cysteine residue in the hinge region is encoded; the C-terminal lysine residue is encoded.
BGpA	1931-2029	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	2103- 2722	pBR322 origin for replication in *E. coli* with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	2877- 3737	Ampicillin resistance for selection in *E. coli*.

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AarI	CACCTGC(4/8)	1	1807	-	
ArsI	(8/13)GACNNNNNNTTYG(11/6)	1	851		
BalI	TGG^CCA	1	3947		MlsI MluNI MscI Msp20I
BcgI	(10/12)CGANNNNNNTGC(12/10)	1	3466		
Bpu10I	CCTNAGC(-5/-2)	1	930		
BsePI	G^CGCGC	1	1182		BssHII PauI PteI
BtrI	CACGTC(-3/-3)	1	1356	-	AjiI BmgBI
CspCI	(11/13)CAANNNNNGTGG(12/10)	1	407		
EagI	C^GGCCG	1	1909		BseX3I BstZI EclXI Eco52I
Eco31I	GGTCTC(1/5)	1	1529		Bso31I BsaI
EcoNI	CCTNN^NNNAGG	1	1487		BstENI XagI
EcoRI	G^AATTC	1	1019		
Esp3I	CGTCTC(1/5)	1	1202		BsmBI
FalI	(8/13)AAGNNNNNCTT(13/8)	1	784		
FspI	TGC^GCA	1	3170		Acc16I AviII NsbI
HindIII	A^AGCTT	1	2052		
NmeAIII	GCCGAG(21/19)	1	3073		
NotI	GC^GGCCGC	1	1908		CciNI
NsiI	ATGCA^T	1	1845		EcoT22I Mph1103I Zsp2I
PfoI	T^CCNGGA	1	1323		
PmeI	GTTT^AAAC	1	1000		MssI
PvuI	CGAT^CG	1	3317		Ple19I BpvUI MvrI
PvuII	CAG^CTG	1	642		
SapI	GCTCTTC(1/4)	1	1877	-	BspQI LguI PciSI
SexAI	A^CCWGGT	1	1645		CsiI MabI
SmaI	CCC^GGG	1	1624		Cfr9I TspMI XmaI
SnaBI	TAC^GTA	1	357		BstSNI Eco105I
SpeI	A^CTAGT	1	18		AhlI BcuI
XhoI	C^TCGAG	1	966		StrI TliI Sfr274I PaeR7I SlaI
AclI	AA^CGTT	2	3175		Psp1406I
		2	3548		
AlwNI	CAGNNN^CTG	2	1252		CaiI PstNI
		2	2468		
BciVI	GTATCC(6/5)	2	2266	-	BfuI BsuI
		2	3793		
BdaI	(10/12)TGANNNNNNTCA(12/10)	2	1223		
		2	2139		
BseRI	GAGGAG(10/8)	2	715		
		2	1440		
BsgI	GTGCAG(16/14)	2	1488	-	
		2	1857		
Bsp1407I	T^GTACA	2	1606		BsrGI BstAUI
		2	3981		
BspMI	ACCTGC(4/8)	2	1659		Acc36I BfuAI BveI
		2	1807		
BsrBI	CCGCTC(-3/-3)	2	1906	-	AccBSI MbiI
		2	3789		
BssSI	CACGAG(-5/-1)	2	2230	-	BauI Bst2BI
		2	3614		
Bsu36I	CC^TNAGG	2	1332		Eco81I AxyI Bse21I
		2	1374		
BtsI	GCAGTG(2/0)	2	3343		
		2	3371		
DrdI	GACNNNN^NNGTC	2	1371		AasI DseDI

		2	2159	
Eam1105I	GACNNN^NNGTC	2	1273	AhdI AspEI BmeRI DriI
		2	2945	
GsuI	CTGGAG(16/14)	2	1062	BpmI
		2	3035	
NcoI	C^CATGG	2	379	Bsp19I
		2	1049	
NdeI	CA^TATG	2	252	FauNDI
		2	2008	
OliI	CACNN^NNGTG	2	1160	AleI
		2	1340	
PflMI	CCANNNN^NTGG	2	641	AccB7I BasI Van91I
		2	1049	
SacI	GAGCT^C	2	583	Ecl136II EcoICRI Eco53kI Psp124BI SstI
		2	1034	
SacII	CCGC^GG	2	740	Sfr303I KspI SgrBI Cfr42I SstII
		2	1434	
TstI	(8/13)CACNNNNNNTCC(12/7)	2	1255	
		2	1514	
VspI	AT^TAAT	2	25	AseI PshBI
		2	3121	
XmnI	GAANN^NNTTC	2	1823	Asp700I MroXI PdmI
		2	3545	

Absent Sites:
AbsI, AflII, AgeI, AjuI, AlfI, AloI, ApaI, AscI, AsuII, AvrII, BaeI, BamHI, BarI, BbvCI, BclI, BlpI, BplI, BsaBI, BsiWI, BsmI, BspEI, BstAPI, BstEII, BstXI, BstZ17I, ClaI, DraIII, Eco47III, EcoRV, FseI, FspAI, HpaI, KflI, KpnI, MauBI, MfeI, MluI, MreI, NaeI, NarI, NheI, NruI, PacI, PasI, PciI, PmaCI, PshAI, PsiI, PspXI, PsrI, PstI, RsrII, SalI, SfiI, SgfI, SgrAI, SgrDI, SphI, SrfI, Sse8387I, StuI, SwaI, Tth111I, XbaI, 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).

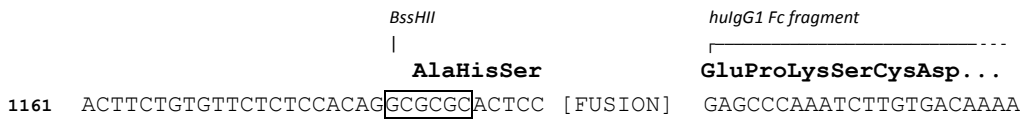
Plasmid Maintenance

Propagation and maintenance of **TGEX-FC** 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 37ºC with agitation. **TGEX-FC** is a derivative 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 37ºC (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-FC** plasmid DNA can be isolated in large quantities.

Cloning into TGEX-FC

In Silico Clone Design

A complete IGHV1-2 leader sequence MDWTWRILFLVAAATGAHS is necessary for secretion of the antibody in the culture supernatant and proper removal of the leader peptide by host proteases. In the following schema, after cutting by BssHII and BsmBI, the end of the leader encoding sequence 5'-CGCGCACTCC will be removed. This short sequence must be included back in the final clone for proper protein maturation and secretion. The BsmBI site will be eliminated during the cloning.



Vector Digestion

BssHII and BsmBI restriction enzymes are fully active at 50ºC and 55ºC, respectively. The double digestion of the vector can be either conducted at 50ºC with both enzymes together or at 55ºC with BsmBI initially alone and together with BssHII after cooling the reaction to 50ºC. Esp3I is an isoshiszomer of BsmBI with an optimal working temperature of 37ºC. Please, consult the documentation of your restriction enzyme provider for optimal double digestion conditions.

Primer Design for Restriction Cloning with the BssHII Site

Oligo1 is an example of primer designed to amplify a VH domain sequence and clone it into the BssHII site. A minimum of 2 nucleotides is recommended to cut BssHII site close to the end (source New Englands BioLabs); these 2 N are followed by the BssHII site GCGCGC and the end of the leader sequence ACTCC encoding the end of the leader peptide; the resulting NNGCGCGCACTCC extension is added 5' to the VH domain primer.

Oligo1 5'-NNGCGCGCACTCC-VH-start

Primer Design for Restriction Cloning with the BsmBI Site

Oligo2 is an example of primer designed to amplify the VH domain from the end of the J region and includes a BsmBI site compatible with **TGEX-FC** cloning site. BsmBI is a type IIS restriction enzyme that cuts outside of its recognition site. BsmBI will 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-bases overhang GAGC on the sense strand. In Oligo2, a BsmBI site situated symmetrically to the vector will generate a complementary overhang. After digestion and ligation, the two BsmBI sites, the one in the vector and the one in Oligo2, will be removed. We added two nucleotides on the 5' end of the primer although a minimum of one nucleotide is recommended to cut BsmBI site close to the end (source New Englands BioLabs).

Oligo2 5'-NNcgtctcNGCTC-JH-end

Alternative to BsmBI Sites

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

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 VH domain in full.

tgex-S3 5'- AGGCGTCTAACCAGTCACAGTC

tgex-R2 5'- CAAAAAATTCCAACACACTATTGC

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 get 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 multiple 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. Similarly, any condition known to boost expression should be carefully tested in your system before being scaled up. We did observe a strong increase in expression in 293 cells upon exposure to sodium valproate (Backliwal 2008).

Appendix

MSDS Information

MSDSs (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, fax, or email us at:

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Fax: 1-858-272-6007 (24 hour)
(Monday – Friday 9:00 AM – 5:00 PM PST)

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