Antibody Design LABORATORIES

TGEX-HChis Expression Vector

INSTRUCTION MANUAL

TGEX-HChis Transient Mammalian Expression Vector

Catalog #: MX005

Version: A1.2 – September 2020

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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™-HChis** vector is designed for the expression of a heavy chain variable region with the constant region of the human IgG1 heavy chain. Expression of full length antibody molecules is achieved by co-transfection with a light chain variable region cloned into the vector **TGEX™-LC**.

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
TGEX-HChis	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-HChis 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

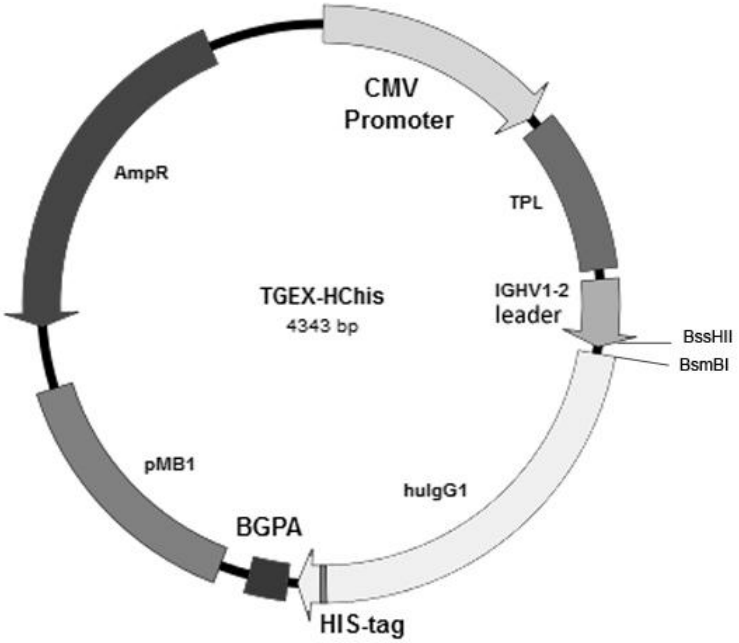
Combination of vectors to desired antibody format

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.

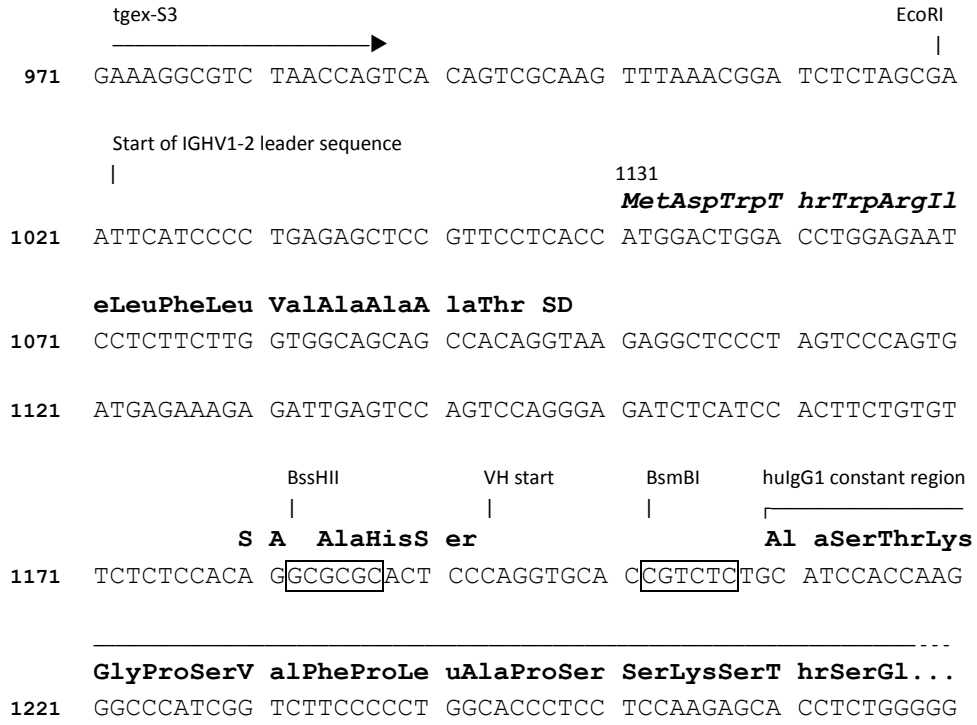
Vector Map

The figure below illustrates the main features of **TGEX-HC** 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 4343 bp.



Cloning Site

Following is an illustration of **TGEX-HChis** 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 CDS	1209-2231	Sequence encoding the human IgG1 heavy chain sequence comprising the CH1, CH2 and CH3 domains with the hinge region. The sequence is intronless and terminated by the HIS tag encoding sequence.
BGpA	2255-2353	Rabbit beta-globin polyadenylation signal sequence.
pMB1 origin	2427- 3046	pBR322 origin for replication in *E. coli* with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992).
TEM1 beta-lactamase	3201- 4061	Ampicillin resistance for selection in *E. coli*.

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AarI	CACCTGC(4/8)	1	2101	-	
AgeI	A^CCGGT	1	1313		AsiGI BshTI CspAI PinAI
ApaI	GGGCC^C	1	1220		Bsp120I PspOMI
ArsI	(8/13)GACNNNNNNTTYG(11/6)	1	851		
BalI	TGG^CCA	1	4271		MlsI MluNI MscI Msp20I
BamHI	G^GATCC	1	2199		
BbvCI	CCTCAGC(-5/-2)	1	1400		
BcgI	(10/12)CGANNNNNNTGC(12/10)	1	3790		
BlpI	GC^TNAGC	1	2227		Bpu1102I Bsp1720I CelII
BsePI	G^CGCGC	1	1182		BssHII PauI PteI
BstEII	G^GTNACC	1	1412		BstPI Eco91I EcoO65I PspEI
BtrI	CACGTC(-3/-3)	1	1650	-	AjiI BmgBI
CspCI	(11/13)CAANNNNNGTGG(12/10)	1	407		
EagI	C^GGCCG	1	2233		BseX3I BstZI EclXI Eco52I
Eco31I	GGTCTC(1/5)	1	1823		Bso31I BsaI
EcoRI	G^AATTC	1	1019		
Esp3I	CGTCTC(1/5)	1	1202		BsmBI
FspI	TGC^GCA	1	3494		Acc16I AviII NsbI
HindIII	A^AGCTT	1	2376		
NarI	GG^CGCC	1	1338		DinI EgeI EheI KasI Mly113I SfoI SspDI
NmeAIII	GCCGAG(21/19)	1	3397		
NotI	GC^GGCCGC	1	2232		CciNI
NsiI	ATGCA^T	1	2139		EcoT22I Mph1103I Zsp2I
PasI	CC^CWGGG	1	1279		
PfoI	T^CCNGGA	1	1617		
PmeI	GTTT^AAAC	1	1000		MssI
PvuI	CGAT^CG	1	3641		Ple19I BpvUI MvrI
PvuII	CAG^CTG	1	642		
SapI	GCTCTTC(1/4)	1	2171	-	BspQI LguI PciSI
SexAI	A^CCWGGT	1	1939		CsiI MabI
SmaI	CCC^GGG	1	1918		Cfr9I TspMI XmaI
SnaBI	TAC^GTA	1	357		BstSNI Eco105I
SpeI	A^CTAGT	1	18		AhlI BcuI
Tth111I	GACN^NNGTC	1	1319		AspI PflFI PsyI
XhoI	C^TCGAG	1	966		StrI TliI Sfr274I PaeR7I SlaI
AclI	AA^CGTT	2	3499		Psp1406I
		2	3872		
AlwNI	CAGNNN^CTG	2	1546		CaiI PstNI
		2	2792		
BciVI	GTATCC(6/5)	2	2590	-	BfuI BsuI
		2	4117		
BdaI	(10/12)TGANNNNNNTCA(12/10)	2	1517		
		2	2463		
Bpu10I	CCTNAGC(-5/-2)	2	930		
		2	1400		
BsgI	GTGCAG(16/14)	2	1782	-	
		2	2151		
Bsp1407I	T^GTACA	2	1900		BsrGI BstAUI
		2	4305		
BspMI	ACCTGC(4/8)	2	1953		Acc36I BfuAI BveI
		2	2101		
BsrBI	CCGCTC(-3/-3)	2	2230	-	AccBSI MbiI
		2	4113		
BssSI	CACGAG(-5/-1)	2	2554	-	BauI Bst2BI
		2	3938		
BstXI	CCANNNNN^NTGG	2	1426		

		2	2216	
BtsI	GCAGTG(2/0)	2	3667	
		2	3695	
DrdI	GACNNNN^NNGTC	2	1665	AasI DseDI
		2	2483	
Eam1105I	GACNNN^NNGTC	2	1567	AhdI AspEI BmeRI DriI
		2	3269	
EcoNI	CCTNN^NNNAGG	2	1289	BstENI XagI
		2	1781	
FalI	(8/13)AAGNNNNNCTT(13/8)	2	784	
		2	1296	
NcoI	C^CATGG	2	379	Bsp19I
		2	1049	
NdeI	CA^TATG	2	252	FauNDI
		2	2332	
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	1728	
TstI	(8/13)CACNNNNNNTCC(12/7)	2	1549	
		2	1808	
VspI	AT^TAAT	2	25	AseI PshBI
		2	3445	
XmnI	GAANN^NNTTC	2	2117	Asp700I MroXI PdmI
		2	3869	

Absent Sites:

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

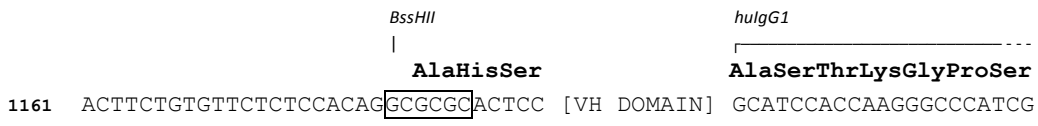
Plasmid Maintenance

Propagation and maintenance of **TGEX-HChis** 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-HChis** 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-HChis** plasmid DNA can be isolated in large quantities.

Cloning into TGEX-HChis

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-HChis** 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 GCAT 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'-NNcgtctcNATGC-JH-end

Removing BsmBI Sites in J Regions

As shown in Table 1, many heavy chain J regions contain a BsmBI site near their 3' end. These sites can be eliminated by modifying the codon usage in the sequence encoding the TVS amino acid sequence. We suggest using GTT instead of GTC for the codon encoding the valine residue; the resulting mismatch will create minimal perturbation and simply adding one extra nucleotide to the J primer is sufficient to ensure strong amplification of the VH domain.

J Region	**3' Sequence**
Human IGHJ1	ctggtcac**cgtctc**ctca g
Human IGHJ2	ctggtcactgtctcctca g
Human IGHJ3	atggtcac**cgtctc**ttca g
Human IGHJ4	ctggtcac**cgtctc**ctca g
Human IGHJ5	ctggtcac**cgtctc**ctca g
Human IGHJ6	acggtcac**cgtctc**ctca g
Mouse mus. IGHJ1*01	acggtcac**cgtctc**ctca g
Mouse mus. IGHJ1*02	acggtcaccgtttcctca g
Mouse mus. IGHJ1*03	acggtcac**cgtctc**ctca g
Mouse mus. IGHJ2*01	actctcacagtctcctca g
Mouse mus. IGHJ2*02	tctctcacagtctcctca g
Mouse mus. IGHJ2*03	agtctcacagtctcctca g
Mouse mus. IGHJ3*01	ctggtcactgtctctgca g
Mouse mus. IGHJ4*01	tcagtcac**cgtctc**ctca g
Rabbit IGHJ1	ctggtcaccatctcttca g
Rabbit IGHJ2	ctggtcac**cgtctc**ctca g
Rabbit IGHJ3	ctggtcac**cgtctc**ctca g
Rabbit IGHJ4	ctggtcac**cgtctc**ctca g
Rabbit IGHJ5*01	ctggtcac**cgtctc**ttca n
Rabbit IGHJ5*02	ctggtcactgtctcttca g
Rabbit IGHJ6	ctcgtcac**cgtctc**ttca g
Translation	T V S S/A

Table I. Occurrence of BsmBI sites on the 3' end of human, mouse musculus and rabbit functional J regions (source IMGT [5]).

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 VH 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).

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.

IMAC Purification

Follow the recommendation of your IMAC resin provider for purification using the HIS tag. The HIS tag located at the C-terminal of the heavy chain can sustain wash in the presence of up to 10 mM imidazole at pH 7.4; leakiness may appear at 20 mM and above.

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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(Monday – Friday 9:00 AM – 5:00 PM PST)

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