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TGEX-HC-hG1[YTE-KF]-Zeo

Mammalian Expression Vector for Fc-Engineered Constant Region of Human IgG1 Heavy Chain
Catalog #: MX044
Version: A1.2 – October 2023
For Research Use Only

Product Information

Contents: 10 µg of TGEX-HC-hG1[YTE-KF]-Zeo plasmid in 20 µl of DNA Conservation Buffer (5 mM Tris-HCl, 0.1 mM EDTA, pH 8.5) (0.5 µg/µl).

Storage: The plasmid is shipped on ice. Upon receipt, store at the plasmid at -20°C.

Quality control: The plasmid was confirmed by sequencing and certified to meet all specifications.

General Product Use

The **TGEX™** vector series is designed for the rapid expression of antibody molecules by transient gene expression in mammalian cells in suspension culture. Transfection of **TGEX™** expression vectors harboring variable region inserts typically yields antibody titers between 50 – 250 mg/L in serum-free conditions in just a few days.

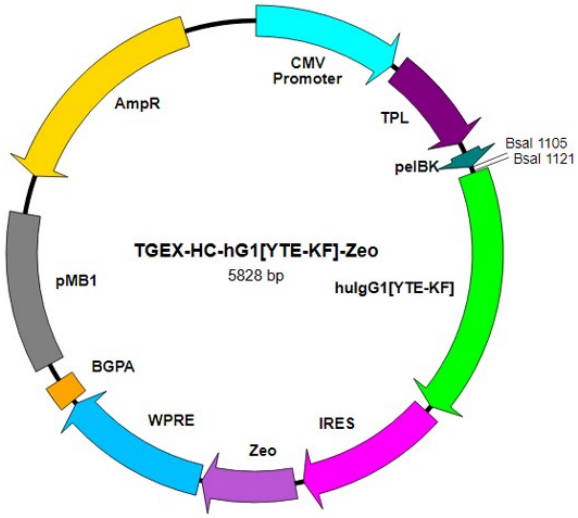
TGEX™-HC family vectors are designed for the expression of an antibody heavy chain variable region with the constant heavy region of an isotype of choice (**Antibody Design Labs** offers many different isotypes in both wild-type and engineered forms, see **Related Products**). Expression of full-length antibodies is achieved with co-transfection with a light chain variable region cloned in to one of the **TGEX™-LC** family vectors.

Antibody Design Labs provides a variety of engineered **TGEX™-HC-hG** vectors, which are designed to express human heavy chains with specific amino acid substitutions in their constant region known to modulate antibody half-life or increase/decrease antibody effector functions (i.e., antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)).

References

1. **Logan J., & Shenk T. 1984.** Adenovirus tripartite leader sequence enhances translation of mRNAs late after infection. *Proc Natl Acad Sci*, *81*(12):3655–9.
2. **Mariati, H. *et al.* 2010.** Evaluating post-transcriptional regulatory elements for enhancing transient gene expression levels in CHO K1 and HEK293 cells. *Protein Expr Purif*, *69*(1):9–15.
3. **Valadon P. *et al.* 2006.** Screening phage display libraries for organ-specific vascular immunotargeting in vivo. *Proc Natl Acad Sci*, 103(2):407–12.
4. **Lin-Chao S. *et al.* 1992**. High copy number of the PUC plasmid results from a ROM/ROP-suppressible point mutation in RNA II. *Mol Microbiol*, 6(22):3385–93.
5. **Vaccaro, C., Zhou, J., Ober, R. *et al*. 2005.** Engineering the Fc region of immunoglobulin G to modulate in vivo antibody levels. *Nat Biotechnol* 23, 1283–1288.

Plasmid Features



- **CMV promoter** and **adenovirus tripartite leader sequence**[1,2] for enhanced expression and translation of transgenes.
- **Composite pelBK leader**[3]: a hybrid between mammalian kappa leader and bacterial leader for secretion of the immunoglobulin chain.
- Double-inverted BsaI **cloning site** optimized for homologous recombination using **FAST-Licase™** (Catalog #: MB101S, MB101L) or restriction enzyme cloning.
- **IRES** sequence for efficient co-expression of selective marker using the same promoter.
- **Zeo**: *Sh ble* gene from *Streptoalloteichus hindustanus* conferring resistance to Zeocin™ for selection of stable transformants in mammalian cell lines.
- **WPR:** Woodchuck hepatitis post-transcriptional regulatory element for enhanced expression of transgenes.
- **BGPA**: Rabbit β-globin polyadenylation signal for efficient polyadenylation.
- **pMB1:** Origin of replication for *E. coli* with a temperature-sensitive high copy-number phenotype.[4]
- **AmpR:** TEM1 β-lactamase gene conferring resistance to ampicillin for selection in *E. coli.*

Specific Features

- **HC-hG1[YTE-KF]:** human IgG1 heavy chain constant region with M252Y, S254T, T256E, H433K, and N434F mutations known to increase antibody serum half-life.[5]

Protocol

Cloning Into TGEX™-HC-hG Vectors

A double-inverted BsaI cloning site separates the pelBK leader peptide sequence from the beginning of the antibody constant region. Cloning into a BsaI-digested TGEX™ vector may be accomplished by two different methods (see below). Both methods result in removal of BsaI sites, which results in the scarless insertion of the antibody variable domain into the plasmid.

Homologous Recombination (Preferred Method):

Homologous recombination is the easiest and most efficient method to seamlessly clone antibody variable regions into TGEX™ vectors. We recommend the **FAST-LICASE™** (Catalog #: MB101S, MB101L) with the following overhangs:

pelBK overhang 5′– CGGCCCAGCCGGCCATGGCA

hIgG1 overhang 5′– GCATCCACCAAGGGCCCATC
(reverse complement for PCR primers)

For synthetic dsDNA constructs, the overhangs are added to each side of the VH domain. For PCR-amplified constructs, primers should contain the overhang followed by the antibody variable region priming area, see **FAST-LICASE™** manual for additional details.

Restriction Cloning (Alternative Method):

Antibody variable regions can be cloned into TGEX™ vectors by restriction cloning by designing primers to amplify variable regions with the required BsaI sites with sufficient complementarity overhangs for in-frame ligation, see examples of compatible oligos below:

Oligo1 5′-NNGGTCTCTGGCA-VH-start

Oligo2 5′-NNGGTCTCGATGC-JH-end

If the antibody variable region insert contains BsaI sites, which prevents cloning using this method, the BsaI sites can be replaced with sites for another type IIS restriction enzyme that generates identical 4-base long 5′ overhangs (e.g., BsmBI).

Recombinant Antibody Production & Purification

Commonly used cell lines (e.g., HEK293, CHO) adapted for culture in suspension and serum-free conditions are recommended. Many transfection reagents designed for transient transfection are commercially available.

We recommend testing transfections conditions with a fluorescent reporter plasmid first (i.e., **TGEX™-eGFP-Zeo**, Cat# MX022) to evaluate transfection efficiency and determine optimal transfection conditions.

Expression of full-length antibodies requires cotransfection with heavy chain (i.e., **TGEX™-HC** family) and light chain (i.e., **TGEX™-LC**) vectors containing variable regions of interest. We recommend starting with a 1:1 light chain to heavy chain ratio during transfection. In our experience, a 2:1 light chain to heavy chain ratio often exhibits an increased expression, although each antibody may require fine tuning of the ratio for optimal expression.

Stably-transfected cells can be produced by selection using Zeocin™. We recommend culturing transfected cells for 6 days before supplementing growth media with 100 µg/ml Zeocin™. Continue passaging cells at regular intervals and densities while maintaining Zeocin™ concentration for an additional 2-3 weeks before increasing selective pressure to up to 1 mg/ml Zeocin™ for an additional 1-2 weeks. Following selection, proceed to single-cell subcloning and analysis of stable transformants for antibody expression.

Antibody production may be assessed by SDS-PAGE, ELISA, or another quantitative assay. Recombinant antibodies may be purified directly from culture supernatant using Protein A/G/L affinity chromatography or coated magnetic beads.

Legal and Disclaimers

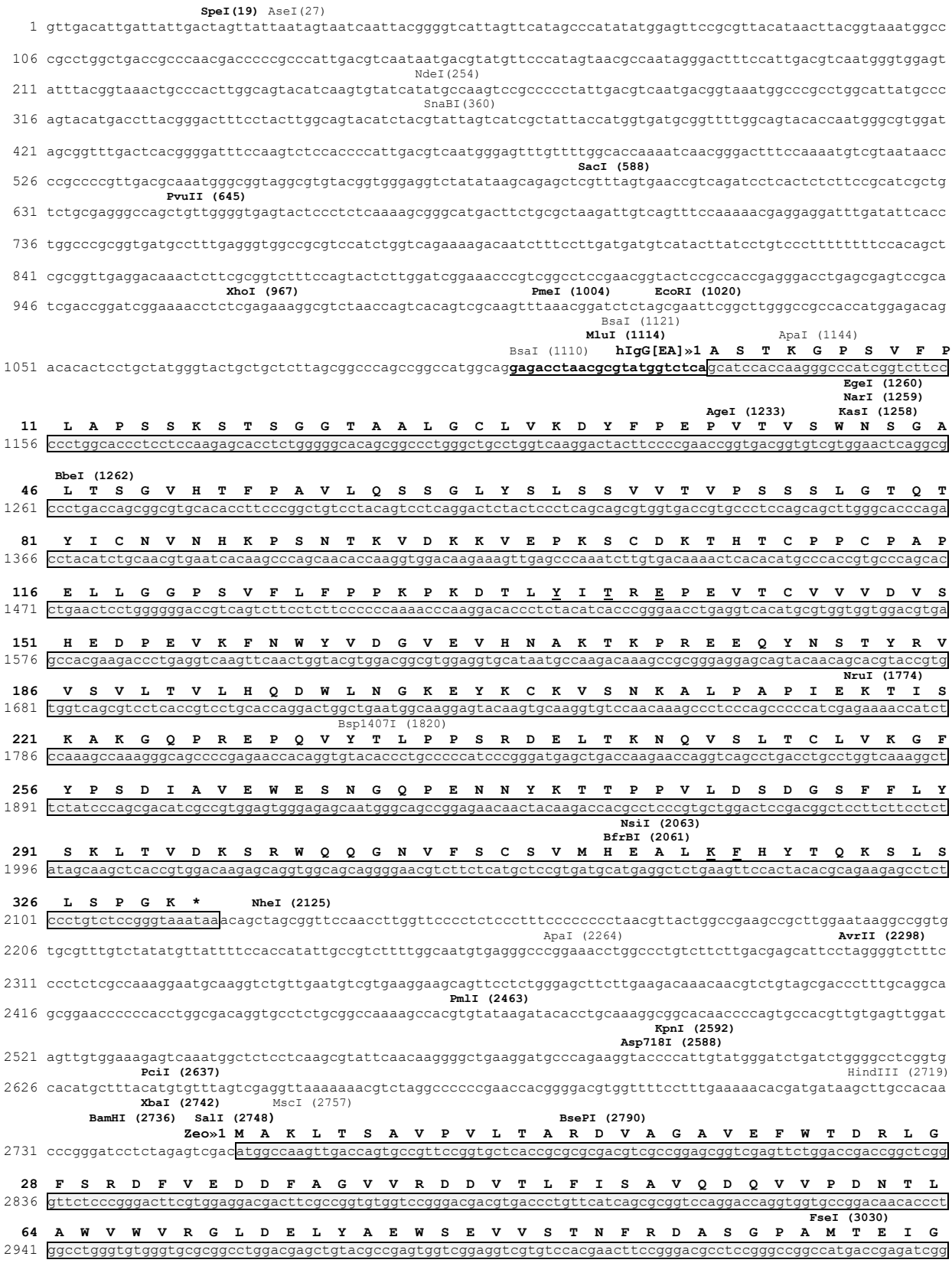
Related Products

VECTOR	CATALOG	ISOTYPE/USE	DESCRIPTION
Antibody Heavy Chain Expression Vectors			
TGEX™-HC-hG1-Zeo	MX026	Human IgG1	Heavy chain expression plasmid for human IgG1
TGEX™-FH-hG1-Zeo	MX023	Human IgG1 CH1	Expression plasmid for human IgG1 Fab fragments
TGEX™-FC-hG1-Zeo	MX025	Human IgG1 Fc	Expression plasmid for 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™-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™-HC-rbG-Zeo	MX039	Rabbit IgG	Heavy chain expression plasmid for rabbit IgG
Fc-engineered Antibody Heavy Chain Expression Vectors			
TGEX™-HC-hG1[EA]-Zeo	MX041	Human IgG1	IgG1 heavy chain with mutated Fc to increase ADCC/CDC
TGEX™-HC-hG1[NA]-Zeo	MX042	Human IgG1	IgG1 heavy chain with mutated Fc for aglycosylated antibody
TGEX™-HC-hG1[LALA-PG]-Zeo	MX043	Human IgG1	IgG1 heavy chain with mutated Fc to reduce ADCC/CDC
TGEX™-HC-hG1[YTE-KF]-Zeo	MX044	Human IgG1	IgG1 heavy chain with mutated Fc to increase serum half-life
TGEX™-HC-hG4[SPLE-PG]-Zeo	MX045	Human IgG4[S228P]	IgG4 heavy chain with mutated Fc to reduce ADCC/CDC
Antibody Light Chain Expression Vectors			
TGEX™-LC-hK-Zeo	MX030	Human Kappa	Light chain expression plasmid for human Kappa
TGEX™-LC-hL-Zeo	MX031	Human Lambda 2	Light chain expression plasmid for human Lambda 2
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™-LC-rbKb4	MX040	Rabbit Kappa b4	Light chain expression plasmid for rabbit Kappa b4
Engineering Vectors			
TGEX™-AC- Zeo	MX020	Any expressions	Universal mammalian expression vector
TGEX™-eGFP- Zeo	MX022	Transfection control	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 scFv-Fc fusion

Examples for Desired Antibody Format

COMBINATION	FORMAT	PURIFICATION METHOD[1]
TGEX™-HC-hG1-Zeo + TGEX™-LC-hK-Zeo	Full-length human or chimeric 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	Human IgG1 Fc fusion	Protein A or G
TGEX™-SCblue-Zeo	Human IgG1 scFv-Fc fusion	Protein A or G

[1]Purification by Protein A, G, and L may require additional testing.


SpeI(19) AseI(27)
NdeI(254)
SnaBI(360)
SacI (588)
PvuII (645)
XhoI (967)
PmeI (1004)
EcoRI (1020)
BsaI (1121)
MluI (1114)
ApaI (1144)
BsaI (1110)
hIgG[EA]»1
EgeI (1260)
NarI (1259)
AgeI (1233)
KasI (1258)
BbeI (1262)
NruI (1774)
Bsp1407I (1820)
NsiI (2063)
BfrBI (2061)
NheI (2125)
ApaI (2264)
AvrII (2298)
PmlI (2463)
KpnI (2592)
Asp718I (2588)
PciI (2637)
HindIII (2719)
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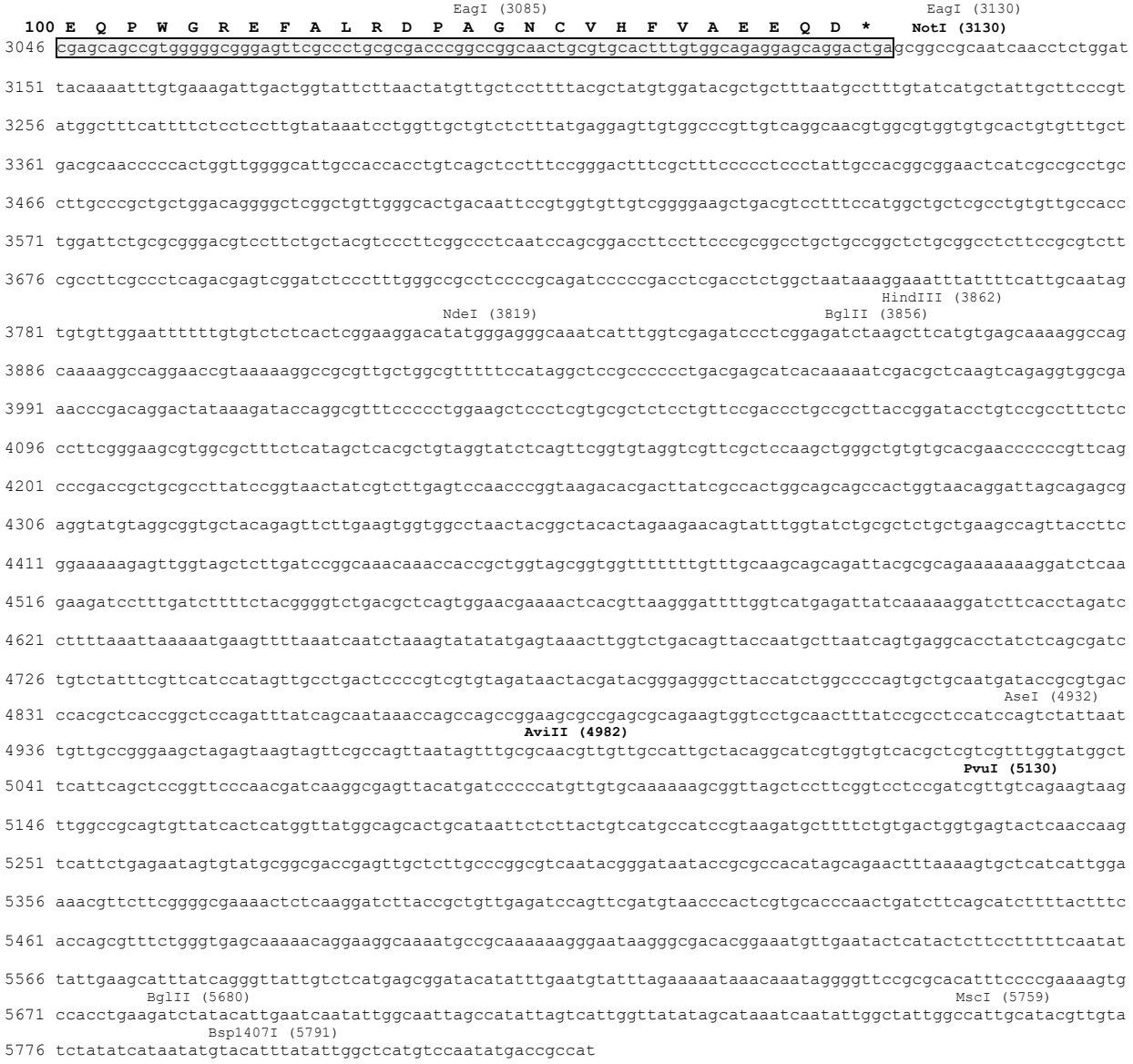
MscI (2757)
BamHI (2736)
SalI (2748)
BsePI (2790)
Zeo»1
FseI (3030)



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