



TGEX™-HC-dG1-Zeo Expression Vector

INSTRUCTION MANUAL

TGEX™-HC-dG1-Zeo Transient Mammalian Expression Vector

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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-dG1-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-dG1-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-dG1-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

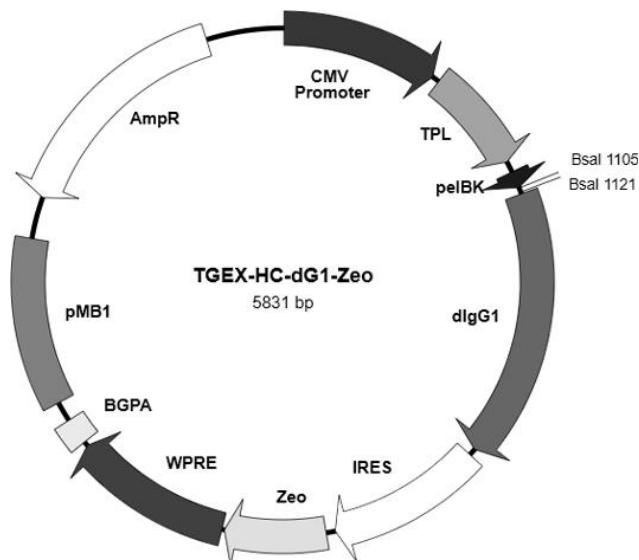
| VECTOR | CATALOG | ISOTYPE | DESCRIPTION |
|---------------------------|---------|-------------------|--|
| TGEX™-HC-hG1[EA]-Zeo | MX041 | Human IgG1 | Human IgG1 heavy chain with increased ADCC/CDC <i>in vitro</i> |
| TGEX™-HC-hG1[NA]-Zeo | MX042 | Human IgG1 | Human IgG1 aglycosylated heavy chain |
| TGEX™-HC-hG1[LALA-PG]-Zeo | MX043 | Human IgG1 | Human IgG1 heavy chain with decreased ADCC/CDC <i>in vitro</i> |
| 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 <i>in vitro</i> |

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 |

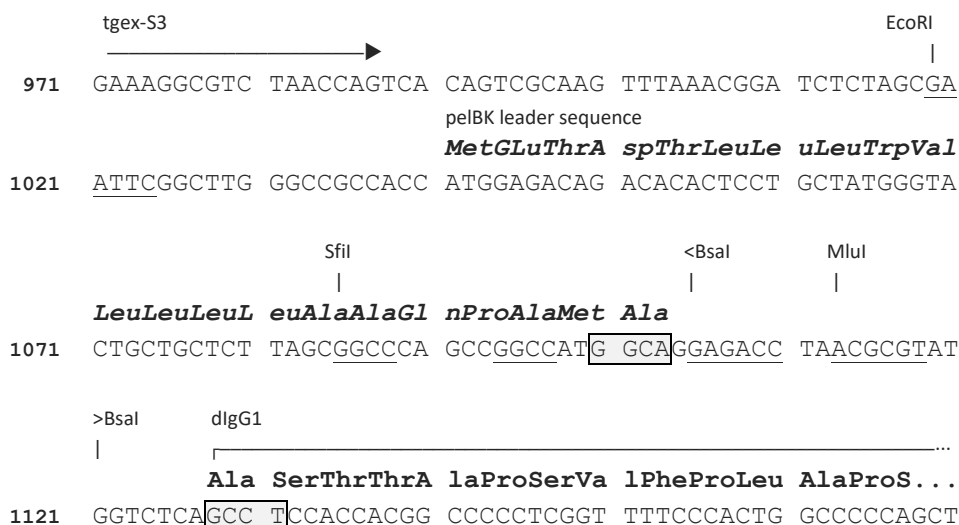
Vector Map

The figure below illustrates the main features of **TGEX™-HC-dG1-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-dG1-Zeo** cloning site from the EcoRI site and onward. The VH domain is inserted in a double inverted Bsal cloning site located between the pelBK leader sequence and the constant regions. The four base pair overhangs after a restriction digestion with Bsal are boxed and grayed.



Feature Table

The features of **TGEX™-HC-dG1-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 IgG1 CDS | 1128-2123 | Sequence encoding the dog IgG1 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 | 2149-2722 | Internal Ribosome Entry Site. |
| Zeo | 2756-3130 | Sh ble gene from <i>Streptoalloteichus hindustanus</i> conferring resistance to Zeocin. |
| WPRE | 3139-3727 | Woodchuck hepatitis virus post-transcriptional regulatory element. |
| BGpA | 3743-3841 | Rabbit beta-globin polyadenylation signal sequence. |
| pMB1 origin | 3915-4534 | pBR322 origin for replication in <i>E. coli</i> with a temperature-sensitive high copy-number phenotype (Lin-Chao 1992). |
| TEM1 beta-lactamase | 5549 - 4689 | Ampicillin resistance for selection in <i>E. coli</i> . |

Restriction Site Summary

| Enzyme | Site | Nb | Position | Strand | Isoschizomers |
|----------|------------------------------|----|----------|--------|------------------------------|
| AlfI | (10/12) GCANNNNNNTGC (12/10) | 1 | 3093 | | |
| AvrII | C^CTAGG | 1 | 2300 | | AspA2I BlnI XmaJI |
| BamHI | G^GATCC | 1 | 2738 | | |
| BbvCI | CCTCAGC (-5/-2) | 1 | 1319 | | |
| BcgI | (10/12) CGANNNNNNTGC (12/10) | 1 | 5278 | | |
| BsePI | G^CGCGC | 1 | 2792 | | BssHII PauI PteI |
| BstAPI | GCANNNN^NTGC | 1 | 1864 | | |
| BstEII | G^GTNACC | 1 | 1544 | | BstPI Eco91I EcoO65I PspEI |
| CspCI | (11/13) CAANNNNNGTGG (12/10) | 1 | 407 | | |
| DrdI | GACNNNN^NNGTC | 1 | 3971 | | AasI DseDI |
| Esp3I | CGTCTC (1/5) | 1 | 1756 | | BsmBI |
| FalI | (8/13) AAGNNNNNCTT (13/8) | 1 | 784 | | |
| FseI | GGCCGG^CC | 1 | 3027 | | RigI |
| FspI | TGC^GCA | 1 | 4982 | | Acc16I NsbI |
| KpnI | GGTAC^C | 1 | 2590 | | Acc65I Asp718I |
| MauBI | CG^CGCGCG | 1 | 2791 | | |
| MluI | A^CGCGT | 1 | 1113 | | |
| NheI | G^CTAGC | 1 | 2127 | | AsuNHI BmtI BspOI |
| NotI | GC^GGCCGC | 1 | 3131 | | CciNI |
| NsiI | ATGCA^T | 1 | 2061 | | EcoT22I Mph1103I Zsp2I |
| OliI | CACNN^NNGTG | 1 | 1547 | | AleI |
| PmaCI | CAC^GTG | 1 | 2463 | | AcvI BbrPI Eco72I PmlI PspCI |
| PmeI | GTTT^AAAC | 1 | 1000 | | MssI |
| PstI | CTGCA^G | 1 | 1296 | | BspMAI |
| PvuI | CGAT^CG | 1 | 5129 | | Ple19I |
| SacI | GAGCT^C | 1 | 583 | | Ecl136II EcoICRI Eco53kI |
| Psp124BI | SstI | | | | |
| SalI | G^TCGAC | 1 | 2750 | | |
| SexAI | A^CCWGGT | 1 | 2919 | | CsiI MabI |
| SfiI | GGCCNNNN^NGGCC | 1 | 1085 | | |
| SgrAI | CR^CCGGYG | 1 | 2869 | | |

| | | | | |
|----------|----------------------------|---|------|-------------------------------|
| SnaBI | TAC^GTA | 1 | 357 | BstSNI Eco105I |
| SpeI | A^CTAGT | 1 | 18 | AhlI BcuI |
| XbaI | T^CTAGA | 1 | 2744 | |
| XcmI | CCANNNNN^NNNNTGG | 1 | 1189 | |
| XhoI | C^TCGAG | 1 | 966 | Sfr274I PaeR7I SlaI |
| AccI | GT^MKAC | 2 | 1407 | FblI XmiI |
| | | 2 | 2750 | |
| AlwNI | CAGNNN^CTG | 2 | 1459 | CaiI PstNI |
| | | 2 | 4280 | |
| ApaI | GGGCC^C | 2 | 1795 | Bsp120I PspOMI |
| | | 2 | 2262 | |
| ArsI | (8/13)GACNNNNNNNTTYG(11/6) | 2 | 851 | |
| | | 2 | 1834 | |
| BalI | TGG^CCA | 2 | 2757 | MlsI MluNI Mox20I MscI Msp20I |
| | | 2 | 5759 | |
| Bpu10I | CCTNAGC(-5/-2) | 2 | 930 | |
| | | 2 | 1319 | |
| BsaAI | YAC^GTR | 2 | 357 | BstBAI Ppu21I |
| | | 2 | 2463 | |
| BsaXI | (9/12)ACNNNNNCTCC(10/7) | 2 | 1721 | - |
| | | 2 | 2992 | |
| BsgI | GTGCAG(16/14) | 2 | 1587 | |
| | | 2 | 1730 | |
| Bsp1407I | T^GTACA | 2 | 1996 | BsrGI BstAUI |
| | | 2 | 5793 | |
| BspHI | T^CATGA | 2 | 4589 | CciI PagI |
| | | 2 | 5597 | |
| BtrI | CACGTC(-3/-3) | 2 | 2690 | - |
| | | 2 | 2888 | AjiI BmgBI |
| | | 2 | 5155 | |
| BtsI | GCAGTG(2/0) | 2 | 5183 | |
| | | 2 | 2507 | AdeI |
| DraIII | CACNNN^GTG | 2 | 3104 | |
| | | 2 | 3087 | BseX3I BstZI EclXI Eco52I |
| EagI | C^GGCCG | 2 | 3132 | |
| | | 2 | 1749 | AhdI BmeRI DriI |
| Eam1105I | GACNNN^NNGTC | 2 | 4757 | |
| | | 2 | 1342 | BstENI XagI |
| EcoNI | CCTNN^NNNAGG | 2 | 1470 | |
| | | 2 | 1019 | |
| EcoRI | G^AATTC | 2 | 1250 | |
| | | 2 | 1343 | - |
| GsuI | CTGGAG(16/14) | 2 | 4847 | BpmI |
| | | 2 | 4113 | BfoI BstH2I |
| HaeII | RGCGC^Y | 2 | 4882 | |
| | | 2 | 2721 | |
| HindIII | A^AGCTT | 2 | 3864 | |
| | | 2 | 252 | FauNDI |
| NdeI | CA^TATG | 2 | 3820 | |
| | | 2 | 2049 | PscI |
| PciI | A^CATGT | 2 | 2639 | |
| | | 2 | 740 | Sfr303I KspI SgrBI Cfr42I |
| SacII | CCGC^GG | 2 | 3639 | |
| | | 2 | 2734 | Cfr9I TspMI XmaI |
| SmaI | CCC^GGG | 2 | 2844 | |
| | | 2 | 1634 | PflFI PsyI |
| Tth111I | GACN^NNGTC | 2 | 1854 | |
| | | 2 | 25 | AseI PshBI |
| VspI | AT^TAAT | 2 | 4933 | |

Absent Sites:

AanI, AbsI, AccIII, AfeI, AflIII, AgeI, AjuI, AloI, Aor13HI, Aor51HI, AscI, AsiGI, AsiSI, AsuII, BaeI, BarI, BclI, BfrI, BlpI, BoxI, BplI, Bpu1102I, Bpu14I, Bsa29I, BsaBI, Bse8I, BseAI, BseCI, BseJI, BshTI, BshVI, BsiWI, Bsp119I, Bsp13I, Bsp1720I, Bsp68I, BspDI, BspEI, BspQI, BspT104I, BspTI, BssNAI, Bst1107I, BstAFI, BstBI, BstPAI, BstXI, BstZ17I, Bsu15I, BsuTUI, BtuMI, ClaI, CpoI, CspAI, CspI, DinI, Eco147I, Eco32I, Eco47III, EcoRV, EgeI, EheI, FbaI, FspAI, HpaI, I-CeuI, I-PpoI, I-SceI, KasI, KflI, Kpn2I, Ksp22I, KspAI, LguI, MfeI, Mly113I, MreI, MroI, MspCI, MunI, NarI, NruI, NspV, PI-PspI, PI-SceI, PacI, PaeI, PalAI, PasI, PceI, PciSI, Pfl23II, PinAI, PluTI, PshAI, PsiI, PspLI, PspXI, PsrI, RgaI, RruI, Rsr2I, RsrII, SapI, SbfI, SdaI, SfaAI, SfoI, SfuI, SgfI, SgrDI, SgsI, SmiI, SphI, SrfI, Sse8387I, SseBI, SspDI, StuI, SwaI, 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 TEGX™ plasmid DNA can be isolated in large quantities.

Cloning into TEGX™-HC-dG1-Zeo

In Silico Design

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

```

                SfiI
                |
    LeuLeuLeuL euAlaAlaGl nProAlaMet Ala
1071 CTGCTGCTCT TAGCGGCCCA GCCGCCATGGCA [VH] Ala SerThrThrA laProSerVa
                |                               |
                dIgG1
                |
                GCC TCCACCACGG CCCCTCGGT

```

Vector Digestion

Bsal 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 TEGX™ 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 Bsal (see kit instructions).

pelBK overhang 5' – CGGCCCAGCCGGCCATGGCA

dIgG1 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-dG1-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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