STOP

Before using this product, please read the Limited Use License statement below:

Important Limited Use License information for pFUSE-Lucia-mIgG1

The purchase of the pFUSE-Lucia-mIgG1 vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

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pFUSE-Lucia-CHIg-mG1

Plasmid featuring the constant region of the mouse IgG1 heavy chain

Catalog # pfuselc-mchg1

For research use only

Version 20K02-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE-Lucia-CHIg-mG1** plasmid provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C and is stable 3 months.
- Resuspended DNA should be stored at -20°C and is stable up to 1 year.
- Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

Materials required for antibody generation & isotype switching

- pFUSE2-CLIg plasmid that features the constant region of the kappa or lambda light chains. pFUSE2-CLIg plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).

- pFUSE-CHIg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin™.

GENERAL PRODUCT USE

pFUSE-CHIg and pFUSE2-CLIg plasmids are designed to change a monoclonal antibody from one isotype to another, therefore, enabling the generation of antibodies with the same antigen affinity but with different effector functions (increased or reduced ADCC and CDC). Furthermore, they can be used to produce entire IgG antibodies from Fab or scFv fragments that are either chimeric, humanized or fully human depending on the nature of the variable region.

pFUSE-CHIg and pFUSE2-CLIg express the constant regions of the heavy (CH) and light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody. Transfection of mammalian cell lines with the recombinant pFUSE-CHIg and pFUSE2-CLIg pair allows to generate an IgG antibody that can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

Features of pFUSE-Lucia-CHIg and pFUSE2-CLIg

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **Lucia luciferase** is a secreted coelenterazine-utilizing luciferase reporter protein with high protein carrier ability. Lucia luciferase serves as a tag to facilitate the detection and quantification of recombinant antibodies. As heavy chains cannot be properly secreted in the absence of light chains, detection of Lucia luciferase in the cell supernatants reflects the presence of full-length antibodies. Lucia luciferase activity in the cell supernatants can be evaluated using QUANTI-Luc™ assay reagent.
- **MCS :** The multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **CMV enh / hFerL prom:** This composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene. This ubiquitous promoter drives the expression of the Zeocin™-resistance gene in mammalian cells.
- **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM2KC is located within an intron and is spliced out in mammalian cells.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

pFUSE-CHIg-mG1 specific features

- **Mouse IgHG1 (IgG1 heavy chain constant region):** When cloning your heavy chain variable region of choice in the MCS, care must be taken to insert the gene in-frame and to preserve the integrity of the heavy chain constant region.
- **Zeo:** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The same resistance gene confers selection in both mammalian cells and *E. coli*.

1. Kim DW. *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23. **2. Takebe Y. *et al.* 1988.** SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72. **3. Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. **4. Yu J. & Russell JE. 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

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E-mail: info@invivogen.com

PROTOCOL

Obtaining VH and VL sequences

To obtain the cDNA sequence of the VH and VL regions from an antibody producing hybridoma, total RNA or mRNA is extracted and reverse transcribed to cDNA. PCR is performed with 5' degenerate primers to anneal to the unknown VH and VL regions and the 3' primers designed to anneal to the "known" CH and CL regions. Alternatively 5' RACE can be used. The resulting amplicons must be sequenced.

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H_2O. Store resuspended plasmid at -20°C.

Cloning into pFUSE-CHIg and pFUSE2-CLIg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSE-Lucia-CHIg-mG1, the constant region of the mouse IgG1 heavy chain is preceded by a multiple cloning site containing four restriction sites: Acc 651, Eco RI, Xho I and Nhe I. The first three restriction sites can be used for insertion of the 5'end of the variable region, taking care to clone in frame with the Lucia® sequence. In pFUSE-Lucia-CHIg-mG1, Nhe I must be used for insertion of the 3'end of the variable region. Nhe I must be reconstituted to maintain the integrity of the constant region. Therefore we recommend to introduce by PCR an Nhe I site at the 3' end of the variable region in frame with the constant region.

Note:

- Lucia® is a secreted protein, therefore, the VH sequence should not include a signal sequence.

- When generating the insert for VL, a Bst API (mouse kappa; pFUSE2-CLIg-mk), or AvrII (mouse lambda; pFUSE2-CLIg-ml1 or pFUSE2-CLIg-ml2) site must be introduced at the 3' end. There is a choice of restriction sites at the 5' end.

Antibody production

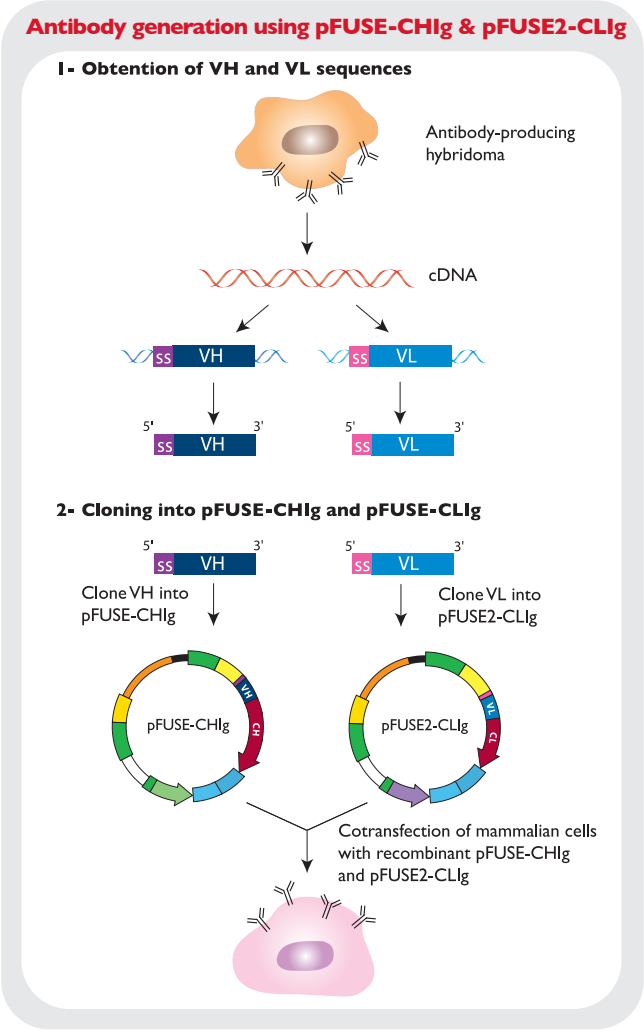
Cotransfect mammalian cells, such as 293 and CHO cells, with the recombinant plasmids pFUSE2-CLIg encoding the light chain and pFUSE-CHIg encoding the heavy chain. Antibody production depends greatly on the ratio of heavy chain and light chain expression. Typically, pFUSE-CHIg to pFUSE2-CLIg ratio of 2:3 is used to cotransfect mammalian cells. Since both plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of plasmids.

OR

Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

Use blasticidin and Zeocin™ to select pFUSE2-CLIg and pFUSE-CHIg respectively.

Antibody production can be analyzed by different techniques including SDS-PAGE, flow cytometry, ELISA, or a bioactivity assay.



Antibody purification

The resulting IgG antibody can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™	rep-qlc1
pFUSE2-CLIg-mk	pfuse2-mclk
pFUSE2-CLIg-ml1	pfuse2-mcll1
pFUSE2-CLIg-ml2	pfuse2-mcll2
pFUSE-Lucia-CHIg-mG2a	pfuselc-mchg2a
pFUSE-Lucia-CHIg-mG2b	pfuselc-mchg2b
pFUSE-Lucia-CHIg-mG3	pfuselc-mchg3
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5
Zeocin™	ant-zn-1

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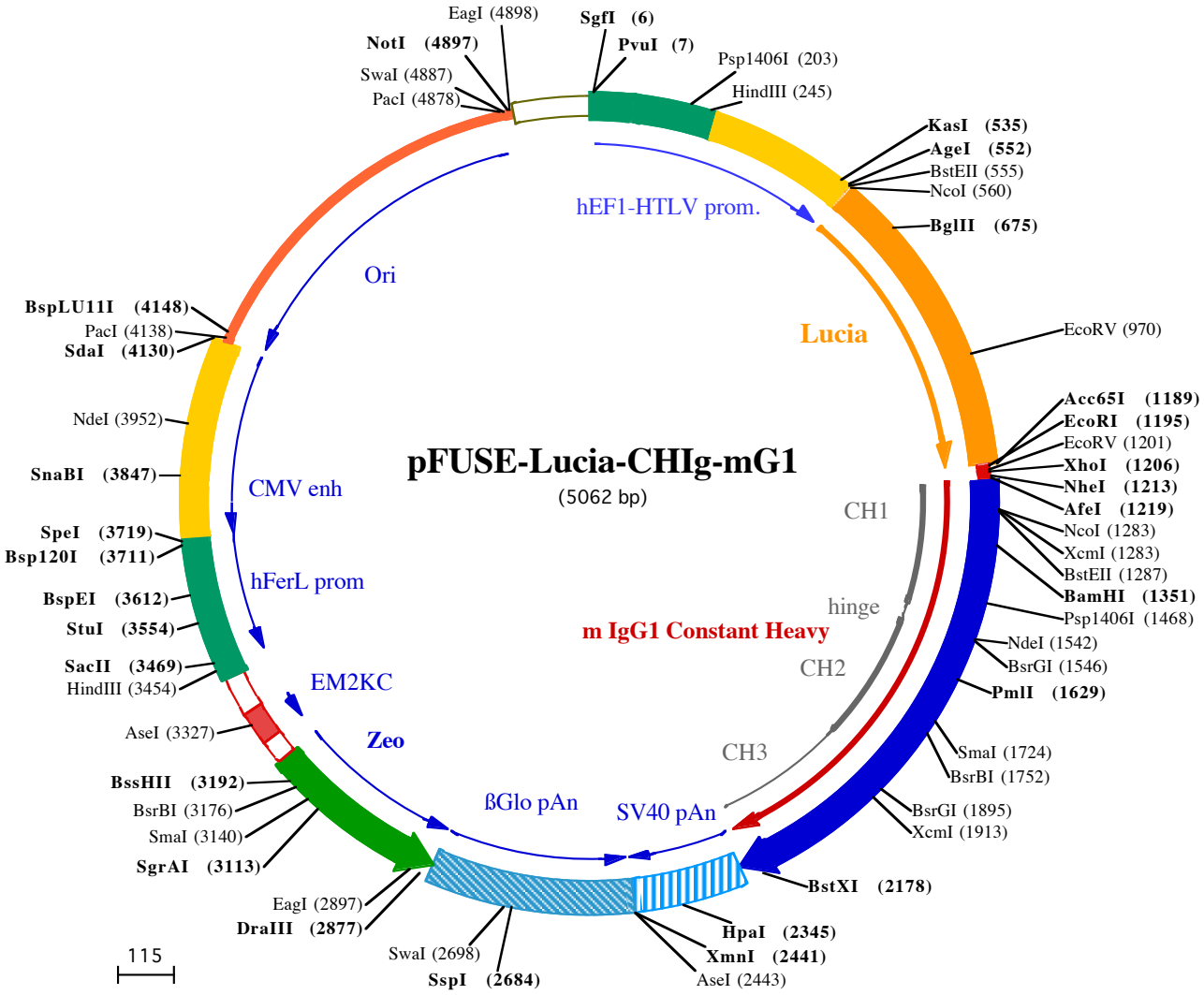
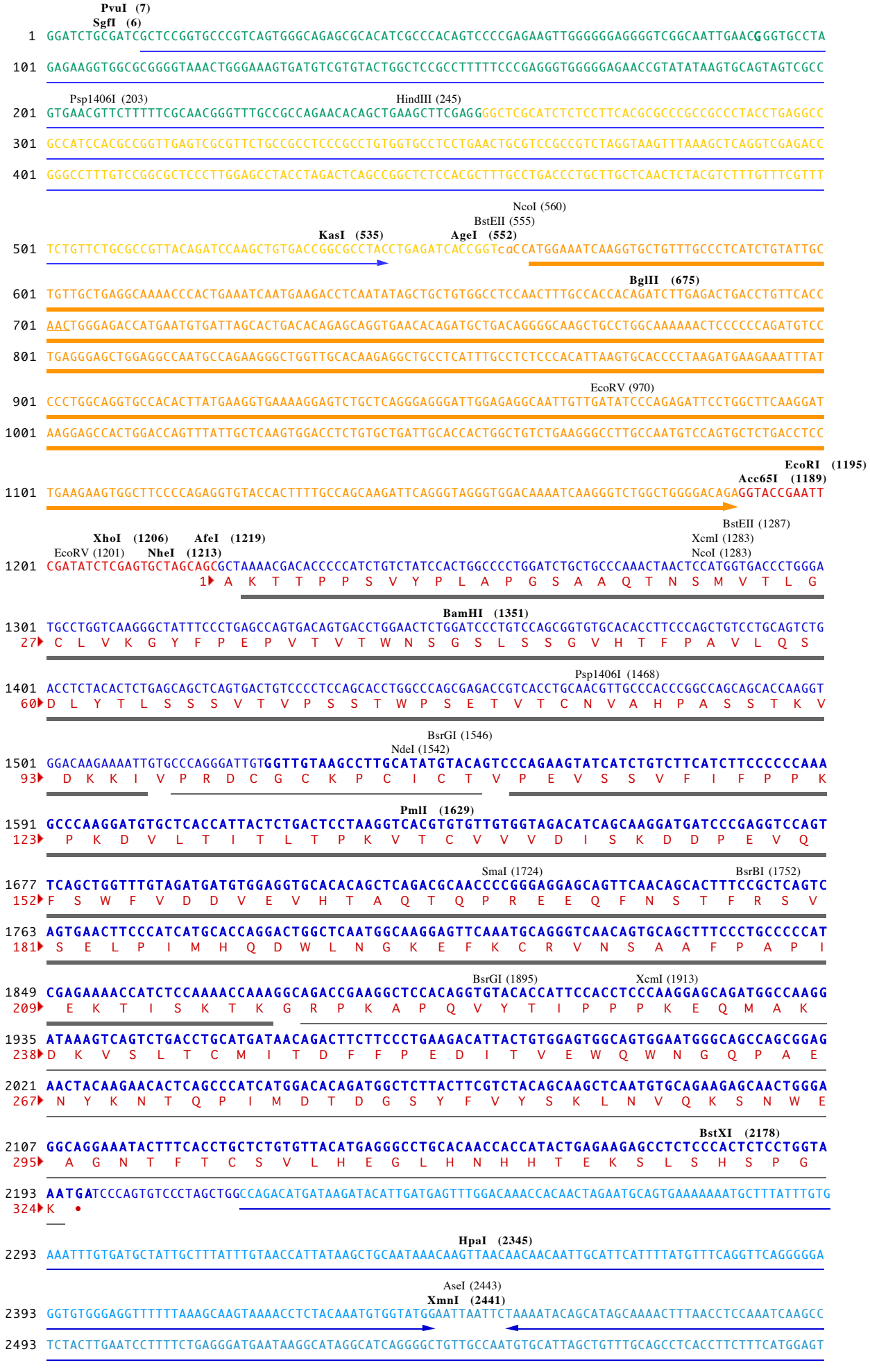
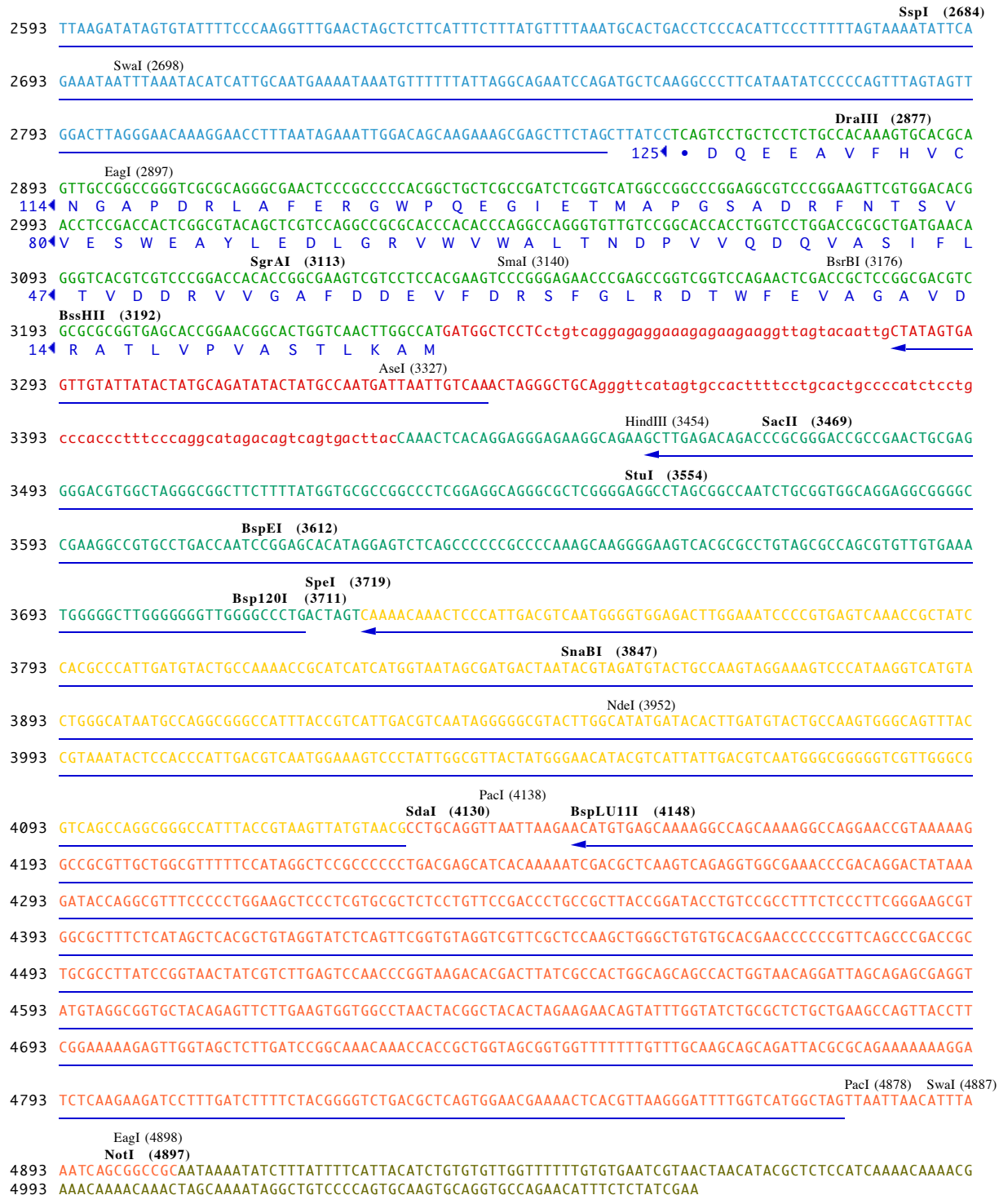
E-mail: info@invivogen.com


pFUSE-Lucia-CHIg-mG1
(5062 bp)
EagI (4898)
NotI (4897)
SwaI (4887)
PacI (4878)
SgfI (6)
PvuI (7)
Psp1406I (203)
HindIII (245)
KasI (535)
AgeI (552)
BstEII (555)
NcoI (560)
BglII (675)
hEF1-HTLV prom.
Ori
Lucia
EcoRV (970)
Acc65I (1189)
EcoRI (1195)
EcoRV (1201)
XhoI (1206)
NheI (1213)
AfeI (1219)
NcoI (1283)
XcmI (1283)
BstEII (1287)
BamHI (1351)
Psp1406I (1468)
NdeI (1542)
BsrGI (1546)
PmlI (1629)
SmaI (1724)
BsrBI (1752)
BsrGI (1895)
XcmI (1913)
BstXI (2178)
HpaI (2345)
XmnI (2441)
AseI (2443)
BspLU11I (4148)
PacI (4138)
SdaI (4130)
NdeI (3952)
SnaBI (3847)
CMV enh
SpeI (3719)
Bsp120I (3711)
hFerL prom
BspEI (3612)
StuI (3554)
SacII (3469)
HindIII (3454)
EM2KC
AseI (3327)
Zeo
BssHII (3192)
BsrBI (3176)
SmaI (3140)
SgrAI (3113)
ßGlo pAn
SV40 pAn
EagI (2897)
DraIII (2877)
SwaI (2698)
SspI (2684)
CH1
hinge
m IgG1 Constant Heavy
CH2
CH3
115


PvuI (7)
SgfI (6)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC
Psp1406I (203)
HindIII (245)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT
NcoI (560)
BstEII (555)
KasI (535)
AgeI (552)
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTcaCCATGGAAATCAAGGTGCTGTTTGCCCTCATCTGTATTGC
BglII (675)
601 TGTTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACTTTGCCACCACAGATCTTGAGACTGACCTGTTCACC
701 AACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAAACTCCCCCCAGATGTCC
801 TGAGGGAGCTGGAGGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTTGCCTCTCCCACATTAAGTGCACCCCTAAGATGAAGAAATTTAT
EcoRV (970)
901 CCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCCTGGCTTCAAGGAT
1001 AAGGAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCC
EcoRI (1195)
Acc65I (1189)
1101 TGAAGAAGTGGCTTCCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGAGGTACCGAATT
BstEII (1287)
XhoI (1206)
AfeI (1219)
XcmI (1283)
EcoRV (1201)
NheI (1213)
NcoI (1283)
1201 CGATATCTCGAGTGCTAGCAGCGCTAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGA
1 A K T T P P S V Y P L A P G S A A Q T N S M V T L G
BamHI (1351)
1301 TGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTG
27 C L V K G Y F P E P V T V T W N S G S L S S G V H T F P A V L Q S
Psp1406I (1468)
1401 ACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGT
60 D L Y T L S S S V T V P S S T W P S E T V T C N V A H P A S S T K V
BsrGI (1546)
NdeI (1542)
1501 GGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAA
93 D K K I V P R D C G C K P C I C T V P E V S S V F I F P P K
PmlI (1629)
1591 GCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGT
123 P K D V L T I T L T P K V T C V V V D I S K D D P E V Q
SmaI (1724)
BsrBI (1752)
1677 TCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTC
152 F S W F V D D V E V H T A Q T Q P R E E Q F N S T F R S V
1763 AGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCAT
181 S E L P I M H Q D W L N G K E F K C R V N S A A F P A P I
BsrGI (1895)
XcmI (1913)
1849 CGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTGTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGG
209 E K T I S K T K G R P K A P Q V Y T I P P P K E Q M A K
1935 ATAAAGTCAGTCTGACCTGCATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAG
238 D K V S L T C M I T D F F P E D I T V E W Q W N G Q P A E
2021 AACTACAAGAACACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGA
267 N Y K N T Q P I M D T D G S Y F V Y S K L N V Q K S N W E
BstXI (2178)
2107 GGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCACTCTCCTGGTA
295 A G N T F T C S V L H E G L H N H H T E K S L S H S P G
2193 AATGATCCCAGTGTCCCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTG
324 K •
HpaI (2345)
2293 AAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGA
AseI (2443)
XmnI (2441)
2393 GGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCC
2493 TCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGT


SspI (2684)
SwaI (2698)
DraIII (2877)
EagI (2897)
SgrAI (3113)
SmaI (3140)
BsrBI (3176)
BssHII (3192)
AseI (3327)
HindIII (3454)
SacII (3469)
StuI (3554)
BspEI (3612)
SpeI (3719)
Bsp120I (3711)
SnaBI (3847)
NdeI (3952)
PacI (4138)
SdaI (4130)
BspLU11I (4148)
PacI (4878)
SwaI (4887)
EagI (4898)
NotI (4897)

Zeocin™

Selection antibiotic for the *Sh ble* gene; cell culture tested

Catalog code: ant-zn-05, ant-zn-1, ant-zn-5, ant-zn-5b

http://www.invivogen.com/zeocin

For research use only

Version 20J14-MM

PRODUCT INFORMATION

Contents

Zeocin™ is supplied a sterile filtered blue solution at 100 mg/ml in HEPES buffer.

- **ant-zn-05:** 5 x 1 ml (500 mg)
- **ant-zn-1:** 10 x 1 ml (1 g)
- **ant-zn-5:** 50 x 1 ml (5 g)
- **ant-zn-5b:** 1 x 50 ml (5 g)

Storage and stability

- Zeocin™ is shipped at room temperature. Upon receipt it should be stored at 4 °C or at -20 °C. Avoid repeated freeze-thaw cycles.
- The expiry date is specified on the product label.
- Zeocin™ is sensitive to high concentrations of acids and bases but a short-term exposure to dilute acids can be tolerated.

Note: *Zeocin™ is stable for 1 month at room temperature.*

QUALITY CONTROL

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Endotoxin level: < 1 EU/mg
- Physicochemical characterization (including HPLC, pH, appearance)
- Cell culture tested: potency validated in Zeocin™-sensitive and Zeocin™-resistant mammalian cell lines
- Non-cytotoxicity of trace contaminants: absence of long-term effects confirmed in Zeocin™-resistant cells

BACKGROUND

Zeocin™ is a selection antibiotic that acts on both eukaryotic and prokaryotic cells. Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*[1-3].

Zeocin™ is the commercial name for a special formulation containing Phleomycin, a copper-chelated glycopeptide antibiotic isolated from a mutant strain of *Streptomyces verticillus*. This antibiotic of the bleomycin family exhibits activity against bacteria, eukaryotic microorganisms, plant and animal cells. Although bleomycin antibiotics perturb plasma membranes, their activity is generally believed to be related to their ability to bind and intercalate DNA thus destroying the integrity of the double helix.

GENERAL GUIDELINES

Successful transfection is influenced by many factors. The health and viability of the cell line, the quality of the nucleic acid used, the transfection reagent, the duration of transfection, and the presence or absence of serum can all play a part.

SAFETY CONSIDERATIONS

Zeocin™ is a harmful compound. Refer to safety data sheet for handling instructions.

CHEMICAL PROPERTIES

Zeocin™ is a mixture of structurally related antibiotics which differ by their terminal amine residues. The antibiotics are in a copper chelated form giving the solution a blue color. Zeocin™ is a labile compound which undergoes irreversible denaturation at high and low pH or in presence of a weak oxidant.

CONDITIONS OF SELECTION

Most cells growing aerobically are killed by 0.5 to 1000 µg/ml Zeocin™. However, the sensitivity of cells is pH dependent, i.e. the higher the pH of culture medium, the greater the sensitivity. Thus the concentration of Zeocin™ required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, the activity of Zeocin™ is reduced by a factor of 2 to 3 in hypertonic media, such as those used for protoplast regeneration. Hence, using low salt medium when possible decreases the amount of Zeocin™ needed.

- *Escherichia coli*

The *Sh ble* gene and the hybrid genes in vectors provided by InvivoGen are driven by synthetic *E. coli* promoters (i.e. EM7). The cells of the common *E. coli* recipient strains (i.e. HB101, DH5α, MC1061) transformed by these vectors are resistant to Zeocin™.

Note: *Do not use an E. coli recipient strain that contains the Tn5 transposable element (i.e. MC1066). Tn5 encodes a bleomycin-resistance gene that will confer resistance to Zeocin™.*

Zeocin-resistant transformants are selected in Low Salt LB agar medium (yeast extract 5 g/L, Tryptone 10 g/L, NaCl 5 g/L, Agar 15 g/L, pH 7.5) supplemented with 25 µg/ml of Zeocin™. Plates containing Zeocin™ are stable for 1 month when stored at 4 °C.

- Mammalian cells

The working concentration of Zeocin™ for mammalian cell lines varies from 50 to 400 µg/ml, in a few cases can be as low as 20 µg/ml or as high as 1000 µg/ml. In a starting experiment we recommend to determine the optimal concentration of Zeocin™ required to kill your host cell line. The killing and the detachment of dead cells from the plate, especially at high cell density, may require a longer time compared to G418. Foci of Zeocin-resistant stable transfectants are usually individualized after 5 days to 3 weeks incubation, depending on the cell line. Suggested concentrations of Zeocin™ for selection in mammalian cells are listed on the next page.

TECHNICAL SUPPORT
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WORKING CONCENTRATIONS

Zeocin™ is normally used at a concentration of 100 µg/ml, a 1000-fold dilution from the stock solution. However, the optimal concentration needs to be determined for your cells. Suggested concentrations of Zeocin™ for selection in some examples of mammalian cells are listed below.

Cell line	Medium	Zeocin™ conc	References
B16 (Mouse melanocytes)	RPMI	20-250 µg/ml	4-6
CHO (Chinese hamster ovarian cells)	DMEM	100-500 µg/ml	4, 7, 8
COS (Monkey kidney cells)	DMEM	100-400 µg/ml	9, 10
HEK293 (Human embryonic kidney cells)	DMEM	100-400 µg/ml	11, 12
HeLa (Human uterine cells)	DMEM	50-100 µg/ml	13, 14
J558L (Mouse melanocytes)	RPMI	400 µg/ml	15
MCF-7 (Human breast adenocarcinoma cells)	DMEM	100-400 µg/ml	16, 17
MEFs (Mouse embryonic fibroblasts)	DMEM	200-400 µg/ml	18, 19
THP-1 (Human monocytes)	RMPI	200 µg/ml	20

REFERENCES

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