pFUSE-CHIg-hM

Plasmid featuring the constant region of the human IgM (allele 3) heavy chain

Catalog # pfuse-hchm3

For research use only

Version 24J04v37-JC

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE-CHIg-hM** 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 feature sthe 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 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 antibody that can be purified from the supernatant using the appropriate affinity chromatography.

Features of pFUSE-CHIg and pFUSE2-CLIg plasmids

- **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.
- **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-hM specific features

- **Human IGHM (IgM allele3 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*.

References:

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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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-CHIg-hM, the constant region of the human IgM heavy chain is preceded by a multiple cloning site containing three restriction sites: EcoRV, XhoI, and NheI. The first two restriction sites can be used for insertion of the 5'end of the variable region including the native signal sequence. If the immunoglobulin signal sequence is unknown, pFUSEss plasmids containing a signal sequence should be used. In pFUSE-CHIg-hM, NheI must be used for insertion of the 3'end of the variable region. NheI must be reconstituted to maintain the integrity of the constant region. Therefore we recommend to introduce by PCR an NheI site at the 3' end of the variable region in frame with the constant region.

Note:

- The 5'end of the variable region should encompass the native ATG initiation codon and the region immediately after which corresponds to the signal sequence. For proper initiation of translation, make sure that your insert contains a Kozak translation initiation sequence upstream of the ATG initiation codon such as (G/A)NNATG.

- When generating the insert for VL, a BsiWI (pFUSE2-CLIg-hk; human kappa), or AvrII (pFUSE2-CLIg-hl2; human lambda 2) 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.

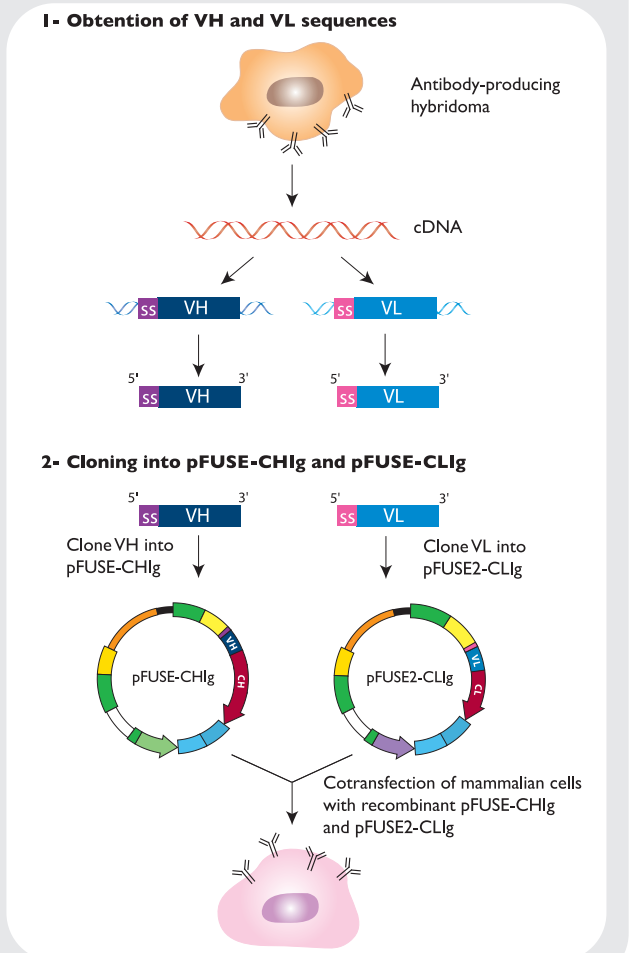
OR

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 generation using pFUSE-CHIg & pFUSE-CLIg



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
pFUSE2-CLIg-hk	pfuse2-hclk
pFUSE2-CLIg-hl2	pfuse2-hcll2
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Zeocin®	ant-zn-1

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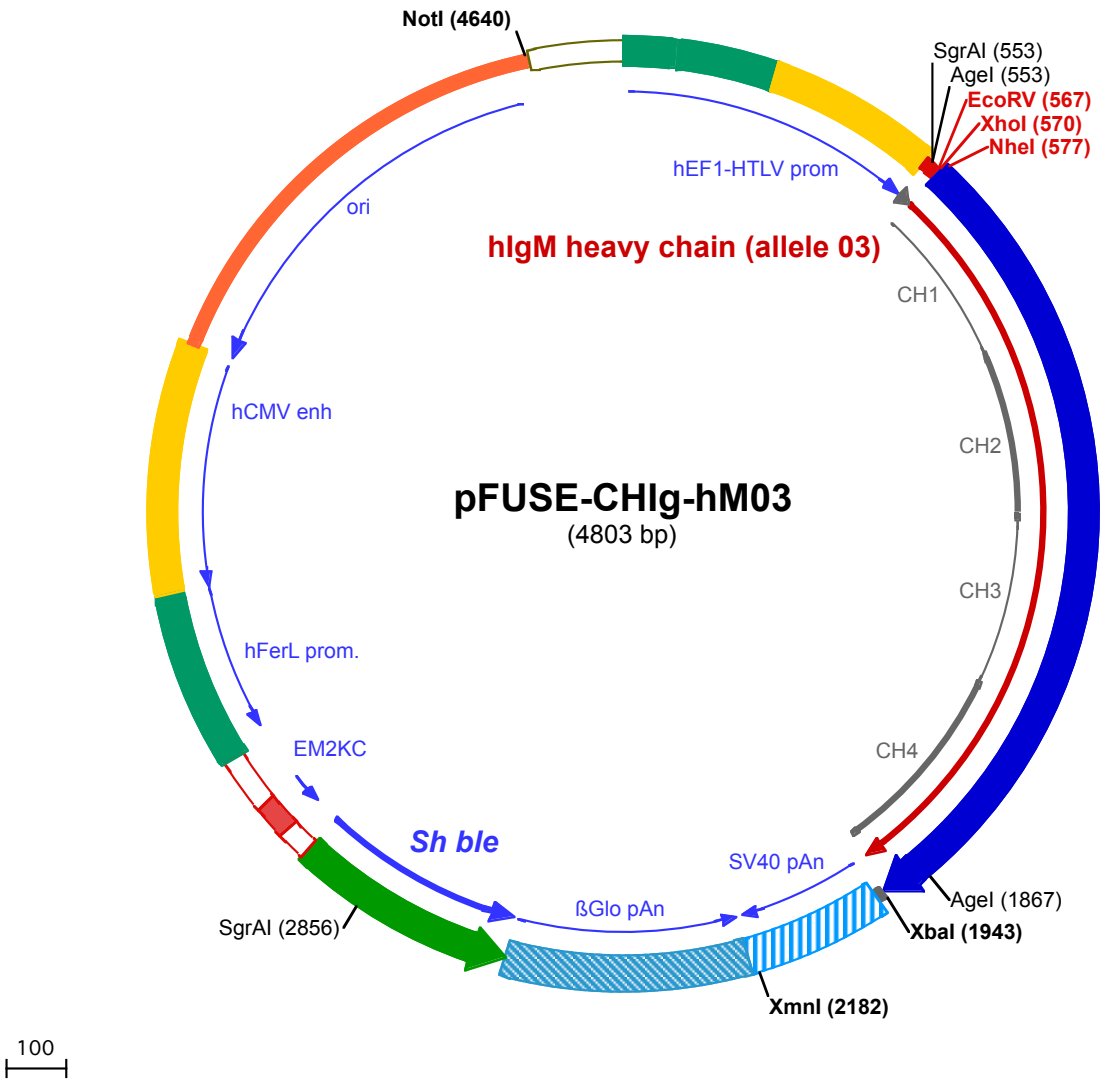
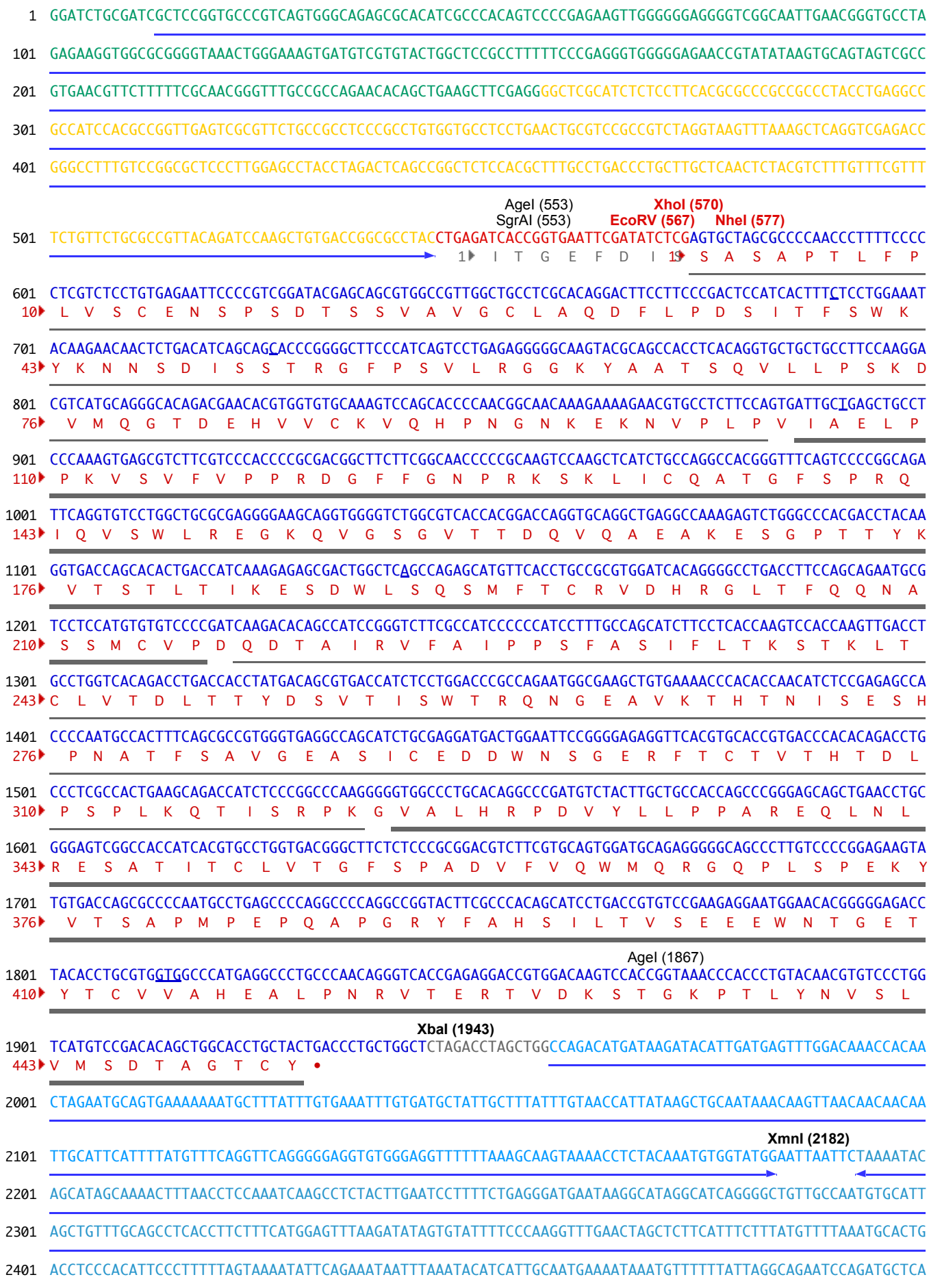
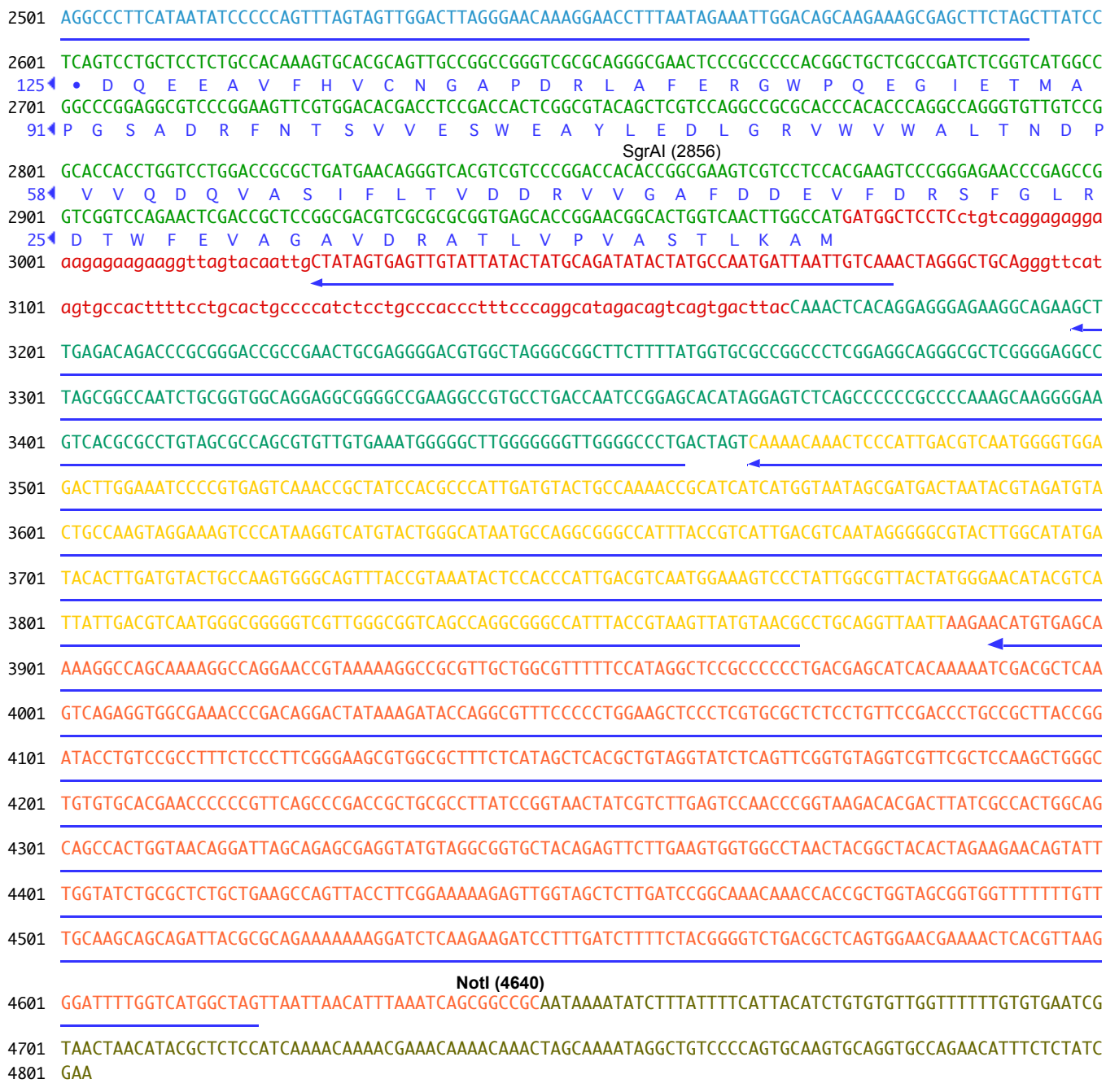

NotI (4640)
SgrAI (553)
AgeI (553)
EcoRV (567)
XhoI (570)
NheI (577)
hEF1-HTLV prom
ori
hIgM heavy chain (allele 03)
CH1
hCMV enh
CH2
pFUSE-CHIg-hM03
(4803 bp)
CH3
hFerL prom.
EM2KC
CH4
Sh ble
SV40 pAn
ßGlo pAn
AgeI (1867)
XbaI (1943)
SgrAI (2856)
XmnI (2182)
100


AgeI (553)
SgrAI (553)
XhoI (570)
EcoRV (567)
NheI (577)
AgeI (1867)
XbaI (1943)
XmnI (2182)


SgrAI (2856)
NotI (4640)

Zeocin®

Selective 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 24G30-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 selective 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 25-50 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.

Note: Zeocin® is a registered trademark of InvivoGen.

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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® concentration	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

1. Drocourt D. *et al.*, 1990. Cassettes of the Streptoalloteichus hindustanus ble gene for transformation of lower and higher eukaryotes to phleomycin resistance. Nucl. Acids. Res. 18: 4009. **2. Gatignol A. *et al.*, 1988.** Bleomycin resistance conferred by a drug-binding protein. FEBS Letters. 230: 171-5. **3. Dumas P. *et al.*, 1994.** The three dimensional structure of a bleomycin resistance protein. Embo J. 242 (5) 595-601. **4. Bouayadi K. *et al.*, 1997.** Overexpression of DNA polymerase beta sensitizes mammalian cells to 2',3' deoxycytidine and 3'-azido-3'-deoxythymidine. Cancer Res. 57: 110-116. **5. Hirose Y. *et al.*, 2012.** Inhibition of Stabilin-2 elevates circulating hyaluronic acid levels and prevents tumor metastasis. PNAS, 109: 4263 - 4268. **6. Fan H. *et al.*, 2012.** Intracerebral CpG immunotherapy with carbon nanotubes abrogates growth of subcutaneous melanomas in mice. Clin Cancer Res.18(20):5628-38. **7. Li F. *et al.*, 1996.** Post-translational modifications of recombinant P-selection glycoprotein ligand-1 required for binding to P- and E- selection. J. Biol. Chem. 271: 3255-3264 . **8. Ogura T. *et al.*, 2004.** Resistance of B16 melanoma cells to CD47-induced negative regulation of motility as a result of aberrant N-glycosylation of SHPS-1. J Biol Chem. 279(14):13711-20. **9. Saxena A. *et al.*, 2002.** H2, the minor subunit of the human asialoglycoprotein receptor, trafficks intracellularly and forms homo-oligomers, but does not bind asialo-orosomucoid. J Biol Chem. 277(38):35297-304. **10. Kanamori A. *et al.*, 2002.** Distinct sulfation requirements of selectins disclosed using cells that support rolling mediated by all three selectins under shear flow. L-selectin prefers carbohydrate 6-sulfation totyrosine sulfation, whereas p-selectin does not. J Biol Chem. 277(36):32578-86. **11. Ahmed *et al.*, 2013.** TRIF-mediated TLR3 and TLR4 signaling is negatively regulated by ADAM15. J Immunol. 190(5):2217-28. **12. Büllesbach EE. & Schwabe C., 2006.** The mode of interaction of the relaxin-like factor (RLF) with the leucine-rich repeat G protein-activated receptor 8. J Biol Chem. 281(36):26136-43. **13. Mesnil M. *et al.*, 1996.** Bystander killing of cancer cells by herpes simplex virus thymidine kinase gene is mediated by connexins. PNAS 93(5):1831-5. **14. Maszczak-Seneczko D. *et al.*, 2013.** UDP-N-acetylglucosamine transporter (SLC35A3) regulates biosynthesis of highly branched N-glycans and keratan sulfate. J Biol Chem. 288(30):21850-60. **15. Cedeno-Laurent F. *et al.*, 2010.** Development of a nascent galectin-1 chimeric molecule for studying the role of leukocyte galectin-1 ligands and immune disease modulation. J Immunol. 185(8):4659-72. **16. Kim HS. *et al.*, 2004.** Insulin-like growth factor-binding protein 3 induces caspase-dependent apoptosis through a death receptor-mediated pathway in MCF-7 human breast cancer cells. Cancer Res. 64(6):2229-37. **17. List HJ. *et al.*, 2001.** Ribozyme targeting demonstrates that the nuclear receptor coactivator AIB1 is a rate-limiting factor for estrogen-dependent growth of human MCF-7 breast cancer cells. J Biol Chem. 276(26):23763-8. **18. Waak J. *et al.*, 2009.** Oxidizable residues mediating protein stability and cytoprotective interaction of DJ-1 with apoptosis signal-regulating kinase 1. J Biol Chem. 284(21):14245-57. **19. MacDonald M. *et al.*, 2007.** The zinc finger antiviral protein acts synergistically with an interferon-induced factor for maximal activity against alphaviruses. J Virol. 81(24):13509-18. **20. Maue A. *et al.*, 2013.** The polysaccharide capsule of Campylobacter jejuni modulates the host immune response. Infect Immun. 81(3):665-72.

RELATED PRODUCTS

Product	Description	Catalog Code
Other selective antibiotics		
Blasticidin	Selective antibiotic for the *bsr* or BSD genes	ant-bl-05
G418	Selective antibiotic for the *neo* gene	ant-gn-1
Hygromycin B Gold	Selective antibiotic for the *hph* gene	ant-hg-1
Puromycin	Selective antibiotic for the *pac* gene	ant-pr-1
Plasmids encoding the *Sh ble* gene		
pMOD2-Zeo	Plasmid encoding a synthetic *Sh ble* gene	pmod2-zeo
pSELECT-zeo-LacZ	LacZ-expression plasmid selectable with Zeocin®	psetz-lacz
pSELECT-zeo-mcs	Expression plasmid selectable with Zeocin®	psetz-mcs

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