

# STOP

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

## **Important Limited Use License information for pFUSE-Lucia-hIgG3**

The purchase of the pFUSE-Lucia-hIgG3 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.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact InvivoGen, 10515 Vista Sorrento Parkway San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.

---

### TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)



# pFUSE-Lucia-CHIg-hG3

Plasmid featuring the constant region of the human IgG3 heavy chain

Catalog # pfuselc-hchg3

For research use only

Version 20K02-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pFUSE-Lucia-CHIg-hG3 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<sup>1</sup> 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<sup>2</sup>. 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<sup>3</sup>.
  - **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<sup>4</sup>.
- ### pFUSE-CHIg-hG3 specific features
- **Human IgHG3 (IgG3 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. *Mol Cell Biol.* 10(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.

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873  
InvivoGen USA (International): +1 (858) 457-5873  
InvivoGen Europe: +33 (0) 5-62-71-69-39  
InvivoGen Hong Kong: +852 3622-3480  
E-mail: [info@invivogen.com](mailto: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  $\mu\text{g}/\mu\text{l}$ , resuspend the DNA in 20  $\mu\text{l}$  of sterile H<sub>2</sub>O. 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-hG3, the constant region of the human IgG3 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<sup>®</sup> sequence. In pFUSE-Lucia-CHIg-hG3, 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<sup>®</sup> is a secreted protein, therefore, the VH sequence should not include a signal sequence.

- When generating the insert for VL, a Bsi WI (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

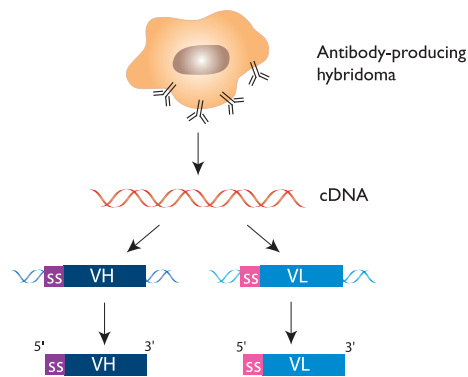
Transfect cells using a transfection agent, such as LyoVec<sup>™</sup>, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

Use blasticidin and Zeocin<sup>™</sup> to select pFUSE2-CLIg and pFUSE-CHIg respectively.

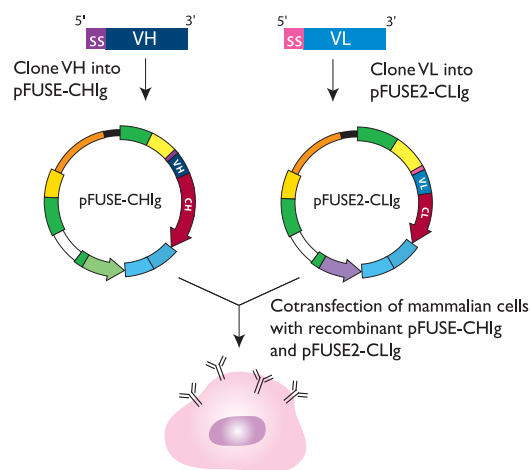
Antibody production can be analyzed by different techniques including SDS-PAGE, flow cytometry, ELISA, or a bioactivity assay.

## Antibody generation using pFUSE-CHIg & pFUSE2-CLIg

### 1- Obtention of VH and VL sequences



### 2- Cloning into pFUSE-CHIg and pFUSE2-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
QUANTI-Luc <sup>™</sup>	rep-qlc1
pFUSE2-CLIg-hk	pfuse2-hclk
pFUSE2-CLIg-hl2	pfuse2-hcll2
pFUSE-Lucia-CHIg-hG1	pfuselc-hchg1
pFUSE-Lucia-CHIg-hG2	pfuselc-hchg2
pFUSE-Lucia-CHIg-hG4	pfuselc-hchg4
LyoVec <sup>™</sup>	lyec-12
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5
Zeocin <sup>™</sup>	ant-zn-1

### TECHNICAL SUPPORT

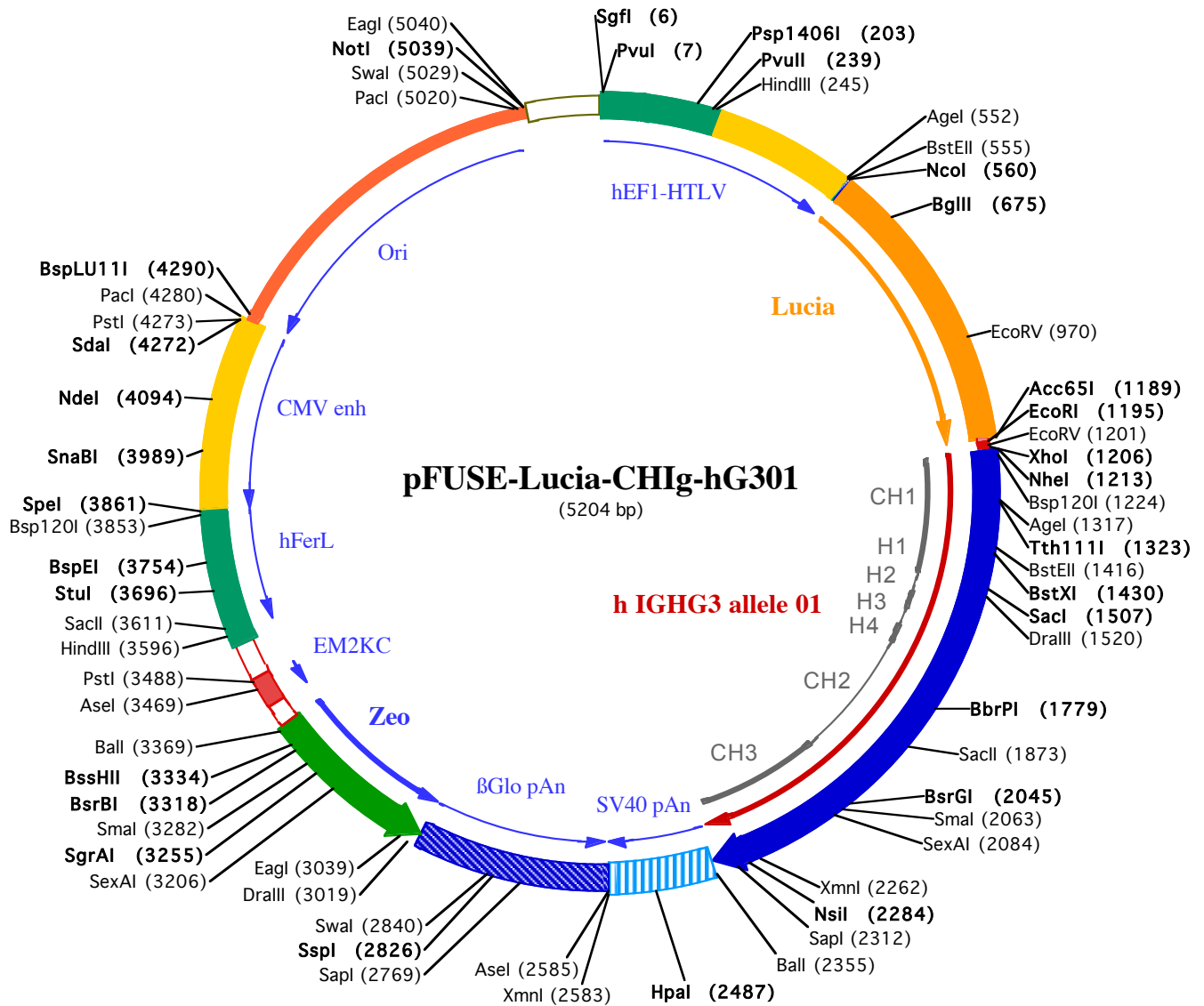
InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)



**PvuI (7)**  
**SgfI (6)**  
 1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGTGCCTA  
 101 GAGAAGTGGCGCGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

**Psp1406I (203)** **HindIII (245)**  
**PvuII (239)**  
 201 GTGAACGTTCTTTTTTCGAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCC  
 301 GCCATCCACGCGGGTTGAGTCGCGTTCGCCGCCCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCGCTAGGTAAGTTTAAAGCTCAGGTCGAGACC  
 401 GGGCCTTTGTCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCCACGCTTTGCTGACCCTGCTTGTCAACTCTACGCTTTGTTTCGTTT

**NcoI (560)**  
**BstEII (555)**  
**AgeI (552)**  
 501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGTcaCCATGGAAATCAAGGTGCTGTTTGCCTCATCTGTATTGC

**BglII (675)**  
 601 TGTGCTGAGGCAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGCCACCACAGATCTTGAGACTGACCTGTTCAACC  
 701 AACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGCAAGTGCCTGGCAAAAACTCCCCCAGATGTCC  
 801 TGAGGGAGCTGGAGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTTGCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAATTTAT

**EcoRV (970)**  
 901 CCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCCAGAGATTCTGGCTTCAAGGAT  
 1001 AAGGAGCCACTGGACAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGTCTGACCTCC

**EcoRI (1195)**  
**Acc65I (1189)**  
 1101 TGAAGAAGTGGCTTCCCCAGAGGTGTACCACCTTTTCCAGCAAGATTGAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGAGGTACCGAATT

**XhoI (1206)**  
**EcoRV (1201)** **NheI (1213)** **Bsp120I (1224)**  
 1201 CGATATCTCGAGTGCTAGCACCAAGGCCCATCGGCTTCCCTGGCGCCTGCTCCAGGACACCTCTGGGGGACAGCGGCCCTGGGCTGCCTGGT  
 1▶ A S T K G P S V F P L A P C S R S T S G G T A A L G C L V

**Tth111I (1323)**  
**AgeI (1317)**  
 1301 AAGGACTACTCCCAAGAACGGTGACGGTGTGGAAGTCAAGGCGCCTGACCGGGCGTGCACACCTTCCGGGTGCTACAGTCTCAGGACTCT  
 30▶ K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L

**BstEII (1416)** **BstXI (1430)**  
 1401 ACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTTGGGACCCAGACCTACACCTGCAACGTGAATCACAAGCCAGCAACACCAAGGTGGACAA  
 63▶ Y S L S S V V T V P S S S L G T Q T Y T C N V N H K P S N T K V D K

**SacI (1507)** **DrallI (1520)**  
 1501 GAGAGTTGAGCTCAAAACCCACTTGGTGACACAACACACATGCCACGGTGCCAGAGCCCAATCTTGTGACACACCTCCCCGTGCCACGGTGC  
 96▶ R V E L K T P L G D T T H T C P R C P E P K S C D T P P P C P R C

1601 CCAGAGCCAAATCTTGTGACACACCTCCCCATGCCACGGTGCCAGAGCCCAATCTTGTGACACACCTCCCCGTGCCAAAGGTGCCAGCACCTG  
 130▶ P E P K S C D T P P P C P R C P E P K S C D T P P P C P R C P A P

**BbrPI (1779)**  
 1701 AACTCTGGGAGACCGTCACTTCTCTTCCCCAAAACCAAGGATACCTTATGATTCCCGGACCCTGAGTCACTGCGTGGTGGTGGACGT  
 163▶ E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V

**SacII (1873)**  
 1801 GAGCCACGAAGACCCCGAGGTCCAGTTCAGTGGTACGTGGACGGCTGGAGGTGCATAATGCCAAGACAAGCCGCGGAGGAGCAGTACAACAGCAGC  
 196▶ S H E D P E V Q F K W Y V D G V E V H N A K T K P R E E Q Y N S T

1901 TTCCGTGTTGTCAGCGTCTCACCGTCTGCACAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTCTCCAACAAGCCCTCCAGCCCCATCG  
 230▶ F R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I

**BsrGI (2045)** **SmaI (2063)** **SexAI (2084)**  
 2001 AGAAAACCATCTCCAAAACCAAGGACAGCCCCGAGAACCACAGGTGTACACCTGCCCCATCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGAC  
 263▶ E K T I S K T K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T

2101 CTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAGCGGGCAGCCGAGAACTACAACACCAGCCTCCCATGCTGGAC  
 296▶ C L V K G F Y P S D I A V E W E S S G Q P E N N Y N T T P P M L D

**XmnI (2262)** **NsiI (2284)**  
 2201 TCCGACGGCTCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACATCTTCTCATGCTCGTGATGCATGAGGCTCTGC  
 330▶ S D G S F F L Y S K L T V D K S R W Q Q G N I F S C S V M H E A L

**SapI (2312)** **BalI (2355)**  
 2301 ACAACCGCTTACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGACAAACCACA  
 363▶ H N R F T Q K S L S L S P G K •

**HpaI (2487)**  
 2401 ACTAGAATGCAGTGAAAAAATGCTTTATTGTGAAATTTGTGATGCTATTGCTTTATTGTAAACCATTATAAGCTGCAATAAACAAAGTTAAACAACAACA

**Asel (2585)**  
**XmnI (2583)**  
 2501 ATTGCAATCATTATGTTTCAGGTTCAAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGGTGTGAATTAATTCTAAATA

2601 CAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCAT

2701 TAGCTGTTTGAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGTTTGAAGTCTTTCATTCTTTATGTTTTAAATGCAT SapI (2769)

2801 GACCTCCACATTCCCTTTTAGTAAATATTAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGCTC SspI (2826) SwaI (2840)

2901 AAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATC

3001 CTCAGTCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTCCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGC DrallI (3019) EagI (3039)  
1254 • D Q E E A V F H V C N G A P D R L A F E R G W P Q E G I E T M A

3101 CGGCCCGGAGGGCTCCCGAAAGTTCTGGACACACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACCCAGGCCAGGGTGTGTCC SmaI (3282)  
924 P G S A D R F N T S V V E S W E A Y L E D L G R V W V W A L T N D

3201 GGCACCACCTGGTCTGGACCGGCTGATGAACAGGGTACGTCGTCGCCGACCACACCGGCGAAGTCTCTCCACGAAGTCCCGGGAGAACCAGGCC SgrAI (3255)  
584 P V V Q D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L R

3301 GGTCCGTCAGAACTCGACCGTCCGGCAGCTCGCGCGGGTGGACCCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagagg Ball (3369)  
254 D T W F E V A G A V D R A T L V P V A S T L K A M

3401 aaagagaagaaggttagtacaattgCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACCTAGGGCTGCAGgggttca AseI (3469) PstI (3488)

3501 tagtgccacttttctgcactgccccatctcctgccaccctttccaggcatagacagtcagtgacttacCAAACCTCACAGGAGGGAGAAGGCAGAAGC HindIII (3596)

3601 TTGAGACAGACCCCGGGACCGCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGCCCTCGGAGGCAGGGCGCTCGGGAGGC StuI (3696)

3701 CTAGCGCCAATCTCGGTGGCAGGAGGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGA BspEI (3754)

3801 AGTCACGCGCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGG SpeI (3861)  
Bsp120I (3853)

3901 AGACTTGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGT SnaBI (3989)

4001 ACTGCCAAGTAGGAAAGTCCATAAGGTGATGTAAGTGGGCATAATGCCAGCGGGCCATTACCCTCATTGACGTCAATAGGGGGCTACTTGGCATATG NdeI (4094)

4101 ATACACTGTAGTACTGCAAGTGGGAGTTACCCTAAATACTCCACCCATTGACGTCAATGAAAAGTCCCTATTGGCGTTACTATGGGAACATACGCTC

4201 ATTATTGACGTCAATGGCGGGGGTCTTGGCGGTCAGCCAGCGGGCCATTTACCCTAAGTTATGTAACGCCCTGCAGGTTAATTAAGAACATGTGAGC PacI (4280)  
PstI (4273)  
SdaI (4272) BspLU11I (4290)

4301 AAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTGTGCTGGCGTTTTTCCATAGGCTCCGCCCTGACGAGCATCACAAAATCGACGCTCA

4401 AGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGCGTTTCCCCTGGAAGTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGCTTACCG

4501 GATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGTTTCTCATAGCTCACGCTGAGGTATCTCAGTTCGGTGTAGGTGTTGCTCCAAGCTGGG

4601 CTGTGTGCACGAACCCCGTTACGCCCAGCGCTGCGCTTATCCGGTAACTATCGTCTTGGTCCAAACCGTAAGACAGACTTATGCCACTGGCA

4701 GCAGCCACTGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTAT

4801 TTGGTATCTGCGCTCTGTAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCCGCTGGTAGCGGTGTTTTTTTGT

4901 TTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAACTCACGTTAA

5001 GGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGCCGCAATAAAATATCTTTATTTTTCATTACATCTGTGTGTTGTTTTTGTGTAATC EagI (5040)  
PacI (5020) SwaI (5029) NotI (5039)

5101 GTAACATAACGCTCTCCATCAAAACAAACGAAACAAACAAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTAT

5201 CGAA

# 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 as 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*<sup>1-3</sup>.

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

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)



## 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)	RPMI	200 µg/ml	20

## REFERENCES

1. Drocourt D. *et al.*, 1990. Cassettes of the *Streptoaloteichus 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 to tyrosine 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. Büllsbach 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
<b>Other selection antibiotics</b>		
Blasticidin	Selection antibiotic for the <i>bsr</i> or <i>BSD</i> genes	ant-bl-05
G418	Selection antibiotic for the <i>neo</i> gene	ant-gn-1
Hygromycin B Gold	Selection antibiotic for the <i>hph</i> gene	ant-hg-1
Puromycin	Selection antibiotic for the <i>pac</i> gene	ant-pr-1
<b>Plasmids encoding the <i>Sh ble</i> gene</b>		
pMOD2-Zeo	Plasmid encoding a synthetic <i>Sh ble</i> gene	pmod2-zeo
pSELECT-zeo-LacZ	LacZ-expression plasmid selectable with Zeocin™	psetz-lacz
pSELECT-zeo-mcs	Expression plasmid selectable with Zeocin™	psetz-mcs

### TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873  
InvivoGen USA (International): +1 (858) 457-5873  
InvivoGen Europe: +33 (0) 5-62-71-69-39  
InvivoGen Hong Kong: +852 3622-3480  
E-mail: [info@invivogen.com](mailto:info@invivogen.com)

