

# pFUSE-CHlg-mG1e3

Plasmid featuring the constant region of an engineered effectorless murine IgG1 heavy chain

Catalog code: pfuse-mchg1e3

<https://www.invivogen.com/pfuse-chig-mg1e3>

For research use only

Version 20F05-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pFUSE-CHlg-mG1e3 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 for 3 months.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year.
- Store Zeocin™ at 4°C or -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-CLlg plasmid that features the constant region of the kappa or lambda light chains. pFUSE2-CLlg plasmids (sold separately, see RELATED PRODUCTS) are selectable with blasticidin.

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

## GENERAL PRODUCT USE

pFUSE-CHlg and pFUSE2-CLlg 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-CHlg and pFUSE2-CLlg 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-CHlg and pFUSE2-CLlg pair allows the generation of 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-CHlg and pFUSE2-CLlg

- **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 a long-lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance the stability of RNA.
- **MCS**: To facilitate cloning of the variable heavy (VH) chain, the multiple cloning site contains the following restriction sites that are compatible with many different enzymes,  
5'- Age I, Eco RI, Eco RV, Xho I, Nhe I, and Eco47 III -3'.
- **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>.
- **pMB1 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** is a composite promoter combining 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 allow the efficient arrest of the transgene transcription<sup>4</sup>.

## pFUSE-CHlg-mG1e3 specific features

- **Murine IgG1e3 (Engineered IgG1 heavy chain constant region)**: When cloning your VH chain 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. The **effectorless mlgG1e3** sequence contains **two point mutations**: D265A (a replacement of aspartic acid by alanine at position 265) and T252M (a replacement of threonine with methionine at position 252). The **D265A mutation** results in the **complete loss of cytolytic effector function**<sup>5</sup>. The **T252M mutation** results in an **increased affinity for Protein A** and enabling efficient purification by affinity chromatography<sup>6</sup>.
- **Sh ble**: 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.* 9(1):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. 5. Baudino L. *et al.*, 2008. Crucial role of aspartic acid at position 265 in the CH2 domain for murine IgG2a and IgG2b Fc-associated effector functions. *J Immunol.* 181(9):6664-9. 6. Nagaoka M. & Akaike T., 2003. Single amino acid substitution in the mouse IgG1 Fc region induces drastic enhancement of the affinity to protein A. *Protein Eng.* 16(4):243-5.

## TECHNICAL SUPPORT

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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<sub>2</sub>O. Store resuspended plasmid at -20°C.

### Cloning into pFUSE-CHlg and pFUSE2-CLlg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSE-CHlg-hG1, the constant region of the murine IgG1e3 heavy chain is preceded by a multiple cloning site containing six restriction sites: Age I, Eco RI, Eco RV, Xho I, Nhe I, and Eco47 III. The first four 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-CHlg-mG1e3, use Eco47III (blunt-end cloning) as the 3' cloning site for the VH to preserve the IgG1 constant amino acid sequence.

*Note: Using NheI as the 3' cloning site will introduce amino acid changes that may not be suitable for some purposes.*

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

*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)NNATGG.*

### Antibody production

Cotransfect mammalian cells, such as 293 and CHO cells, with the recombinant plasmids pFUSE2-CLlg encoding the light chain and pFUSE-CHlg encoding the heavy chain. Antibody production depends greatly on the ratio of heavy chain and light chain expression. Typically, pFUSE-CHlg to pFUSE2-CLlg 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

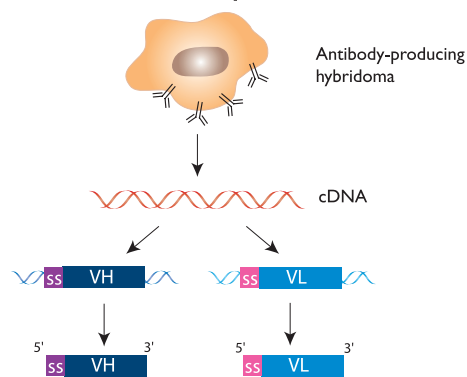
Transfect cells using a transfection agent, such as **LyoVec™**, with the plasmid coding for light chain and select the best clone. Following the selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

Use **blasticidin** and **Zeocin™** to select pFUSE2-CLlg and pFUSE-CHlg respectively.

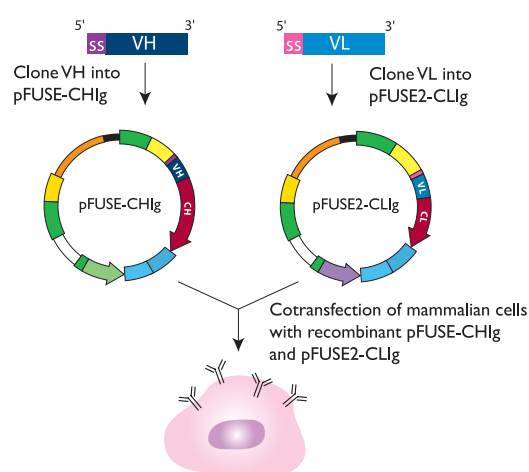
Antibody production can be analyzed by different techniques including SDS-PAGE, flow cytometry, ELISA, or a bioactivity assay.

## Antibody generation using pFUSE-CHlg & pFUSE-CLlg

### 1- Obtention of VH and VL sequences



### 2- Cloning into pFUSE-CHlg and pFUSE2-CLlg



### 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	Description	Cat. Code
pFUSE2-CLlg-mK	Murine κ light chain plasmid	pfuse2-mclk
pFUSE2-CLlg-ml1	Murine λ1 light chain plasmid	pfuse2-mcll1
pFUSE2-CLlg-ml2	Murine λ2 light chain plasmid	pfuse2-mcll2
pFUSE-CHlg-mG1	Murine IgG1 heavy chain plasmid	pfuse-mchg1
pFUSE-CHlg-mG2a	Murine IgG2a heavy chain plasmid	pfuse-mchg2a
pFUSE-CHlg-mG2b	Murine IgG2b heavy chain plasmid	pfuse-mchg2b
pFUSE-CHlg-mG3	Murine IgG3 heavy chain plasmid	pfuse-mchg3
LyoVec™	Transfection reagent	lyec-12
Protein L/Agarose	For IgA and IgG purification	gel-protl-2
Protein G/Agarose	For IgG purification	gel-agg-5
Zeocin™	Selection antibiotic	ant-zn-1

For the complete list of products in our plasmid collection designed for isotype switching, please visit <https://www.invivogen.com/pfuse>.

### TECHNICAL SUPPORT

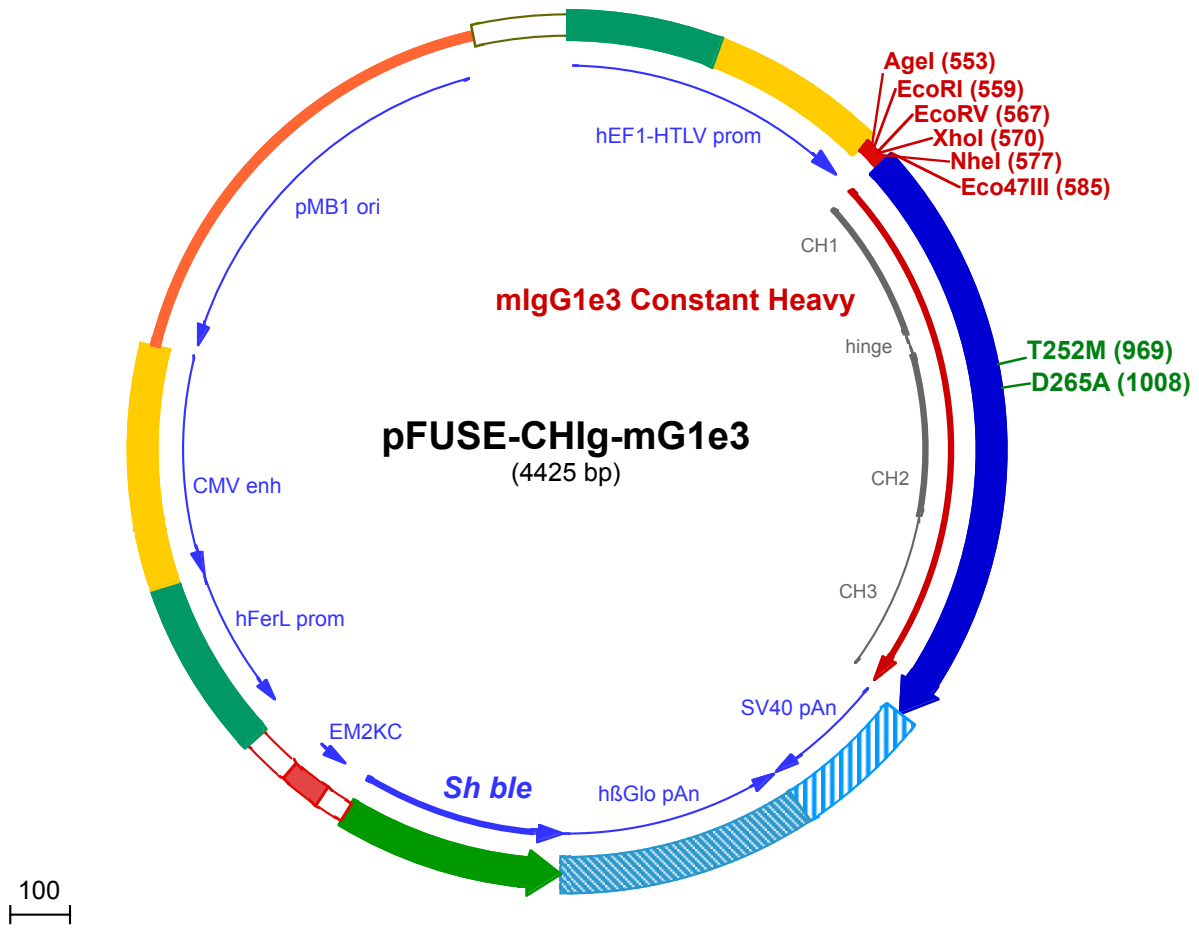
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1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGAGAGCGCACATGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA  
101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC  
201 GTGAACGTTCTTTTTGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCAGAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACCTGAGGCC  
301 GCCATCCACGCCGTTGAGTCCGCTTCTGCCGCTCCCGCTGTGGTGCTCTGAAGTGCCTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC  
401 GGGCCTTTGTCCGGCGCTCCCTTGAGAGCTACCTAGACTCAGCCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT  
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGCGCCTACCTGAGATCACCGGTGAATTCGATATCTCGAGTGTAGCAGCGCTAAAACGACACCC  
EcoRI (559) XhoI (570) Eco47III (585)  
AgeI (553) EcoRV (567) NheI (577)  
1▶ A K T T P  
601 CCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCAAAATAACTCCATGGTGACCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAG  
6▶ P S V Y P L A P G S A A Q T N S M V T L G C L V K G Y F P E P V T  
701 TGACCTGGAAGTCTGGATCCCTGTCAGCGGTGTGCACACCTCCAGCTGTCTGACGTCTGACCTTACACTCTGAGCAGCTCAGTGACTGCCCTC  
39▶ V T W N S G S L S S G V H T F P A V L Q S D L Y T L S S S V T V P S  
801 CAGCACCTGGCCAGCGAGACCTCACCTGCAACGTTGCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGATTGTGGTTGTAAG  
72▶ S T W P S E T V T C N V A H P A S S T K V D K K I V P R D C G C K  
901 CCTGTCATATGTACAGTCCAGAAGTATCATCTGTCTTCTATCTTCCCCCAAAGCCAAAGGATGTGCTGATGATTACTCTGACTCCTAAGGTCACGTGTG  
106▶ P C I C T V P E V S S V F I F P P K P K D V L M I T L T P K V T C  
1001 TTGTGGTAGCCATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGAGGAGCA  
139▶ V V V A I S K D D P E V Q F S W F V D D V E V H T A Q T Q P R E E Q  
1101 GTTCAACAGCACTTTCGCTCAGTCAAGTCCATCATGCACAGGACTGGCTCAATGGCAAGGAGTCAAATGCAGGGTCAACAGTGCAGCTTTC  
172▶ F N S T F R S V 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  
1201 CCTGCCCCATCGAGAAAACATCTCCAAAACAAAGGCAGACCGAAGGCTCCGAGGTGTACACCATTCCACCTCCAAGGAGCAGATGGCCAAGGATA  
206▶ P A P I 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 D  
1301 AAGTCAGTCTGACCTGCATGATAACAGACTTCTCCCTGAAGACATTACTGTGGAGTGGCAGTGAATGGGCAGCCAGCGGAGAAGTACAAGAAGTCA  
239▶ 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 N Y K N T Q  
1401 GCCATCATGGACACAGATGGCTTACTTCGTCTACAGCAAGCTCAATGTGAGAAGAGCAACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTA  
272▶ P I M D T D G S Y F V Y S K L N V Q K S N W E A G N T F T C S V L  
1501 CATGAGGGCTGCACAACCACATACTGAGAAGAGCCTCTCCACTCTCTGGTAAATGATCCAGTGTCCCTAGCTGGCCAGACATGATAAGATACATT  
306▶ H E G L H N H H T E K S L S H S P G K •  
1601 GATGAGTTTGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCA  
1701 ATAAACAAGTTAAACAACAATTGCATTCAATTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGG  
1801 TATGGAATTAATTCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCA  
1901 GGGGCTGTTGCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATAGTGTATTTTCCCAAGGTTTGAAGTCTTTCATT  
2001 TCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTA  
2101 TTAGGCAGAATCCAGATGCTCAAGGCCCTTATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAAATAGAAATTTGGACAGCAAG  
2201 AAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCTCTGCCACAAAGTGCACGAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCCGCCCCACGGCTG  
125▶ • 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  
2301 CTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACACGACTCCGACCCTCGCGGTACAGCTCGTCCAGGCCGCGCACCCAC  
99▶ E G I E T M A 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  
2401 ACCAGGCCAGGGTGTGTCCGGCACCACCTGGTCTGGACCGCGTGTGAACAGGGTCACTCGTCCCGGACCACACCGGCGAAGTCTGCTCCACGA  
65▶ V W A L T N D 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  
2501 AGTCCCGGAGAACCCGAGCCGTCGGTCCAGAAGTCCGACCGTCCGGCAGCTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGAT  
32▶ D R S F G L R 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

2601 GGCTCCTCctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTC  
2701 AACTAGGGCTGCAgggttcatagtgccacttttcctgcactgcccatctctgccaccctttcccaggcatagacagtcagtgacttacCAAACTCA  
2801 CAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGGACCGCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCGCCGCCCTCGG  
2901 AGGCAGGGCGCTCGGGAGGCCCTAGCGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCC  
3001 CCCGCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTC  
3101 CCATTGACGTCAATGGGGTGGAGACTTGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTTGATGTAAGTCCAAAACCGCATCATATGGTAATAG  
3201 CGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAAT  
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3401 GTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGACG  
3501 GTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAG  
3601 CATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCCTG  
3701 TTCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTA  
3801 GGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTGAGCCGACCGCTGCGCCTTATCCGTAACCTATCGTCTTGAGTCCAACCCGGTAAGA  
3901 CACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACG  
4001 GCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCCACCGC  
4101 TGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAG  
4201 TGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTG  
4301 TGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCA  
4401 GGTGCCAGAACATTTCTCTATCGAA

# 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

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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)	RPMI	200 µg/ml	20

## REFERENCES

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