

# pFUSEss-CHIg-mG2b

Plasmid featuring the constant region of the mouse IgG2b heavy chain and the IL2 signal sequence

Catalog # pfusess-mchg2b

For research use only

Version 20J28-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pFUSEss-CHIg-mG2b 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

- pFUSE2ss-CLiG plasmid that features the constant region of the kappa or lambda light chains. pFUSE2ss-CLiG plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).
- pFUSEss-CHIg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin™.

## GENERAL PRODUCT USE

pFUSE-CLiG and pFUSE-CHIg 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 pFUSEss-CHIg and pFUSE2ss-CLiG plasmids

- **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.
- **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.
- **IL2 ss:** The human IL2 signal sequence contains 20 amino acids (MYRMQLLSIALSLALVTNS) and share common characteristics with signal peptides of other secretory proteins. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the immunoglobulin chain.
- **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>.

## pFUSEss-CHIg-mG2b specific features

- **Mouse IgHG2b (IgG2b 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. *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

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## PROTOCOL

### Obtaining VH and VL sequences

The antibody sequence can be obtained by phage display or from an antibody producing hybridoma. 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 pFUSEss-CHlg and pFUSE2ss-CLlg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSEss-CHlg-mG2b, the constant region of the mouse IgG2b heavy chain is preceded by a multiple cloning site containing four unique restriction sites: Eco RI, Eco RV, Nhe I and Eco47III. Using EcoRI as the 5' cloning site ensures that the cloned VH will follow the hIL2 signal sequence without unwanted additional amino-acids. In pFUSE-CHlg-mG2b, use Eco47III (blunt-end cloning) as the 3' cloning site for the VH in order to preserve the IgG2b 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.

### Choice of strategies for the transfection

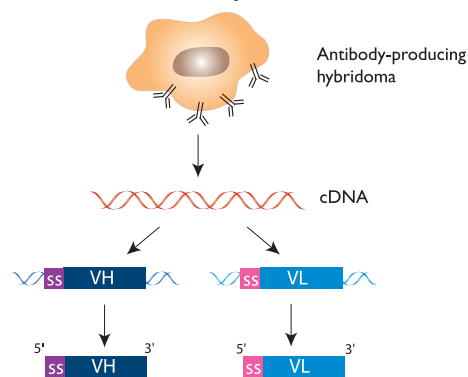
Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

OR

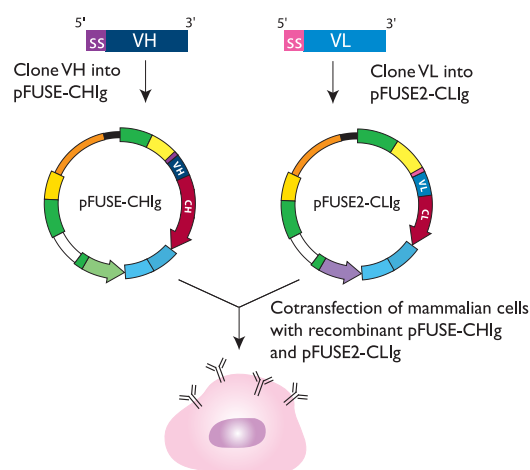
A cotransfection can be performed with the plasmid coding for the light chain and the plasmid coding for the heavy chain. Since the pFUSE2-CLlg and pFUSE-CHlg plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of pFUSE2-CLlg and pFUSE-CHlg plasmids. We recommend using a ratio of 3:2 of pFUSE2-CLlg:pFUSE-CHlg plasmids. pFUSE2-CLlg plasmids feature the constant region of the mouse kappa, lambda 1, or lambda 2 light chain. pFUSE2-CLlg plasmids are selectable with blasticidin. pFUSE-CHlg plasmids are selectable with Zeocin™.

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

### 1- Obtention of VH and VL sequences



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



To check for production of your antibody after transfection, you may take an aliquot of growth medium and perform SDS-PAGE, protein-specific ELISA, or the bioactivity assay of choice to determine that your cells are producing your antibody of interest.

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

## RELATED PRODUCTS

Product	Catalog Code
pFUSE2ss-CLlg-mk	pfuse2ss-mclk
pFUSE2ss-CLlg-ml1	pfuse2ss-mcll1
pFUSE2ss-CLlg-ml2	pfuse2ss-mcll2
pFUSEss-CHlg-hG1	pfuse2ss-mchg1
pFUSEss-CHlg-hG2a	pfuse2ss-mchg2a
pFUSEss-CHlg-hG3	pfuse2ss-mchg3
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5
Zeocin™	ant-zn-1

### TECHNICAL SUPPORT

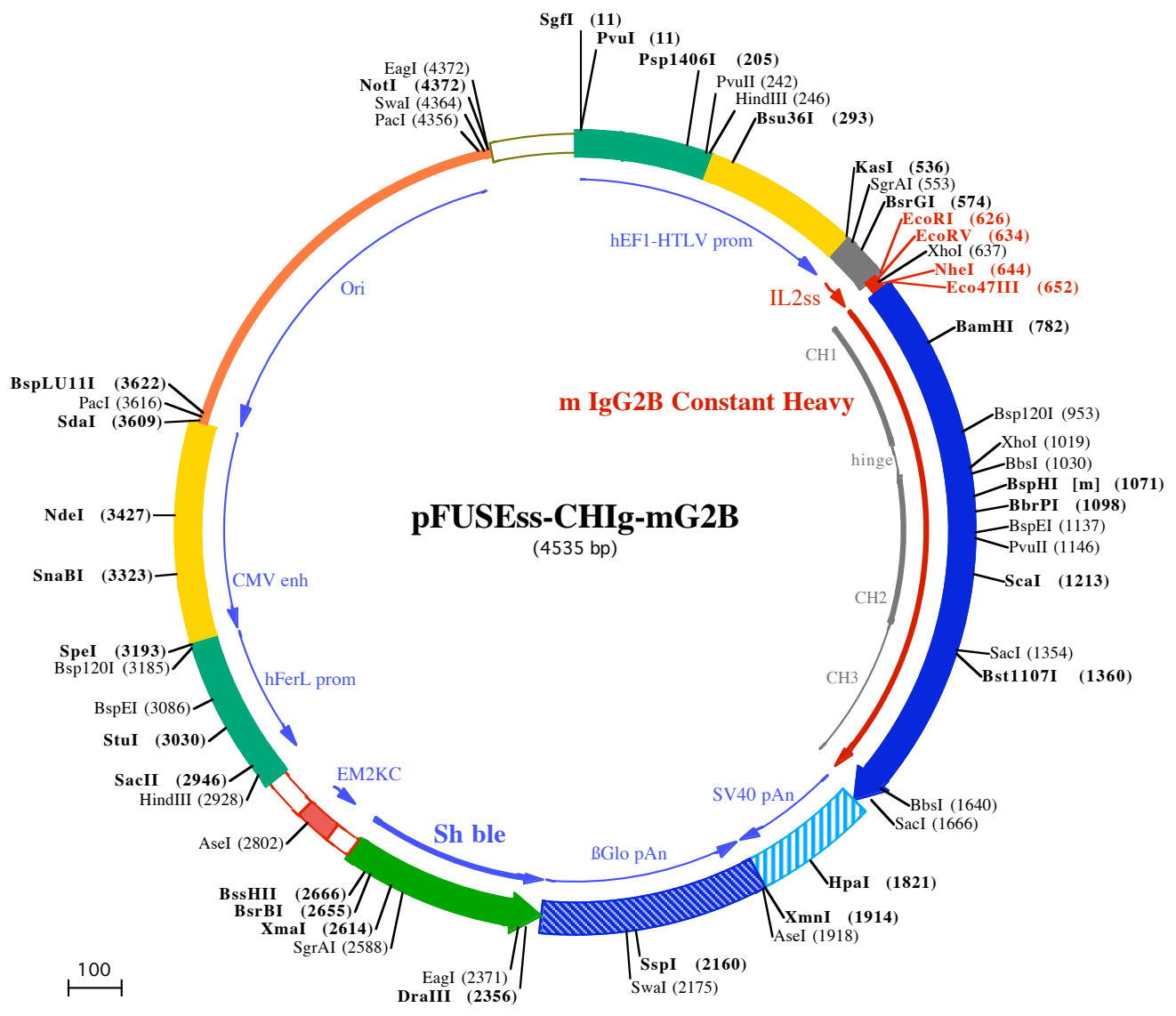
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**PvuI (11)**  
**SgfI (11)**  
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA  
101 GAGAAAGTGGCGCGGGTAAACTGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

**Psp1406I (205)** **HindIII (246)** **PvuII (242)** **Bsu36I (293)**  
201 GTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTACAGCGCCCGCCGCCCTACCTGAGGCC  
301 GCCATCCACGCGGGTTGAGTGCAGTTCGCCGCTCCCGCCTGTGGTGCTCTCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC  
401 GGGCCTTTGTCCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

**KasI (536)** **SgrAI (553)** **BsrGI (574)**  
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGGCGCTACCTGAGATCACCGGCGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGCA  
1 M Y R M Q L L S C

**EcoRV (634)** **Eco47III (652)**  
**EcoRI (626)** **XhoI (637)** **NheI (644)**  
601 TTGCACTAAGTCTTGCACTTGTCACGAATTCGATATCTCGAGTCTAGCAGCGCTAAAACAACACCCCATCAGTCTATCCACTGGCCCTGGGTGTGGA  
1 I A L S L A L V T N S 1 S S A K T T P P S V Y P L A P G C G

**BamHI (782)**  
701 GATACAACCTGGTTCTCTGTGACTCTGGGATGCCTGGTCAAGGGCTACTTCCCTGAGTCAGTACTGTGACTTGGAACTCTGGATCCCTGTCCAGCAGT  
19 D T T G S S V T L G C L V K G Y F P E S V T V T W N S G S L S S S  
800 GTGCACACCTTCCAGCTCTCCTGCAGTCTGGACTCTACACTATGAGCAGCTCAGTACTGTCCCTCCAGCAGCTGGCAAGTCAGACCGTCACCTGCA  
52 V H T F P A L L Q S G L Y T M S S S V T V P S S T W P S Q T V T C

**Bsp120I (953)**  
900 GCGTTGCTACCCAGCCAGCAGCACCGTGGACAAAAAAGTTGAGCCAGCGGGCCATTTCAACAATCAACCCCTGCTCCATGCAAGGAGTGTCA  
85 S V A H P A S S T T V D K K L E P S G P I S T I N P C P P C K E C H

**XhoI (1019)** **BbsI (1030)** **BspHI [m] (1071)** **BbrPI (1098)**  
1000 CAAATGCCAGCTCTAACCTCGAGGTGGACCATCCGCTTTCATCTTCCCTCCAATATCAAGGATGTACTCATGATCTCCCTGACACCCCAAGGTCAG  
118 K C P A P N L E G G P S V F I F P P N I K D V L M I S L T P K V T

**PvuII (1146)** **BspEI (1137)**  
1100 TGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGACGCTCGGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAG  
152 C V V V D V S E D D P D V R I S W F V N N V E V H T A Q T Q T H R

**ScaI (1213)**  
1200 AGGATTACAACAGTACTATCCGGTGGTCACTGCCCTCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTCAAATGCAAGGTCAACAACAAGA  
185 E D Y N S T I R V V S A L P I Q H Q D W M S G K E F K C K V N N K D

**SacI (1354)** **Bst1107I (1360)**  
1300 CCTCCATCACCCATCGAGAGAACCATCTCAAAAATTAAGGGCTAGTCAGAGCTCCACAAGTATACATCTTGCCGCCACCAGCAGAGCAGTTGTCAGG  
218 L P S P I E R T I S K I K G L V R A P Q V Y I L P P P A E Q L S R

1400 AAAGATGTCAGTCTCACTTGCTGGTGGTGGGCTTCAACCTGGAGACATCAGTGTGGAGTGGACCAGCAATGGGCATACTGAGGAGAACTACAAGGACA  
252 K D V S L T C L V V G F N P G D I S V E W T S N G H T E E N Y K D

1500 CCGCACCAGTCTCGACTCTGACGGTCTTACTTTCATATACAGCAAGCTCGATATAAAAACAAGCAAGTGGGAGAAAACAGATTCTTCTCATGCAACGT  
285 T A P V L D S D G S Y F I Y S K L D I K T S K W E K T D S F S C N V

**BbsI (1640)** **SacI (1666)**  
1600 GAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCGGTCTCCGGGTAAGTCAAGCTCAGCACCACAAAGCCTAGCTGGCCAGACATGA  
318 R H E G L K N Y Y L K K T I S R S P G K •

1700 TAAGATACATTGATGAGTTTGGACAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCAT

**HpaI (1821)**  
1800 TATAAGCTGCAATAAACAAGTTAAACAACAATTGCATTCTTTTATGTTTCAGGTTGAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTC

**AseI (1918)** **XmnI (1914)**  
1900 TACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTGAAATCCTTTTCTGAGGGATGAATAAGG  
2000 CATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGACGCTCACCTCTTTTCATGGAGTTAAGATATAGTGTATTTCCCAAGGTTTGAAC

**SspI (2160)** **SwaI (2175)**  
2100 AGCTTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATA

2200 AATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAT

2300 TGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCGCGCCGGGTCGCGCAGGGCGAACTCCCGC  
125 D Q E E A V F H V C N G A P D R L A F E R

2400 CCCACGGCTGCTCGCCGATCTCGGTTCATGCGCCGCGGAGGCGTCCCAGGAGTTCTGTGGACACGACCTCCGACCACTCGGGGTACAGCTCGTCCAGGC  
102 G W P Q 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

2500 CGCGCACCCACACCAGGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCGTGTGAAACAGGGTCACGTCGTCGCGACCAACCGGCGAAGTC  
69 R V W 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

2600 GTCCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGTCAGAACTCGACCGCTCCGGCGACGTCGCGCGGGTGGACCCGGAACGGCACTGGTCAAC  
36 D E V F 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

2700 TTGGCATGATGGCTCCTCctgtcaggagaggaagaagaaggtagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAAT  
24 K A M

2800 GATTAATTGTCAAACCTAGGGCTGCAgggttcattagtgccacttttctgcaactgccccatctcctgccaccctttccaggcatagacagtcaagtact

2900 tacCAAACCTACAGGAGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGACCCGCGAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGCG  
HindIII (2928) SacII (2946)

3000 CCGGCCCTCGGAGGCAGGGCGCTCGGGGAGGCC TAGCGCCAATCTGCGGTGGCAGGAGGCGGGGCCAAGGCCGTGCTGACCAATCCGGAGCACATAG  
StuI (3030) BspEI (3086)

3100 GAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGGTGGGGCCCTGACTAGTC  
Bsp120I (3185) SpeI (3193)

3200 AAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCAT

3300 CATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGCATAATGCCAGGCGGGCCATTTACCGTCA  
SnaBI (3323)

3400 TTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCCAAGTGGGCGTTTACCCTAAATACTCCACCCATTGACGTCAATGGAAG  
NdeI (3427)

3500 TCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAGTTATGT

3600 AACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAAGGCCAGCAAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCC  
Pacl (3616) SdaI (3609) BspLU11I (3622)

3700 CCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGTACCAGGCGTTTTCCCTGGAAGCTCCCTCGT

3800 GCGCTCTCTGTTCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGAGGTATCTC

3900 AGTTCGGTGTAGGCTGTTGCTCCAAGTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCA

4000 ACCCGTAAGACACGACTTATCGCACTGGCAGCAGCCACTGTTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTG

4100 GCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAA

4200 CAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTCGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGT

4300 CTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCA  
Pacl (4356) SwaI (4364) EagI (4372) NotI (4372)

4400 TTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCA

4500 GTCAAGTGCAGGTGCCAGAACATTTCTTATCGAA

# 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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