

pFUSE2-CLIg-hk

Plasmid featuring the constant region of the human immunoglobulin kappa light chain

Catalog # pfuse2-hclk

For research use only

Version 20J15-MM

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE2-CLIg-hk plasmid provided as lyophilized DNA.
- 2 x 1 ml blasticidin at 10 mg/ml

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable up to 1 year.

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.
- pFUSE-CHlg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin™ (sold separately, see RELATED PRODUCTS).

GENERAL PRODUCT USE

pFUSE-CHlg 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-CHlg 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-CHlg 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 pFUSE2-CLIg and pFUSE-CHlg plasmids

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². 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 mRNA³.
- **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 blasticidin-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 transcription⁴.

pFUSE2-CLIg-hk specific features

- **Human IgKC (Ig Kappa Light constant domain):** When cloning your kappa light chain variable region of choice in the MCS, care must be taken to preserve the integrity of the kappa light chain constant region and reading frame.
- **Bsr (blasticidin resistance gene):** Resistance to blasticidin is conferred by the bsr gene from *Bacillus cereus*. 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

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 μl of sterile H₂O. 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 pFUSE2-CLIg-hk, the constant region of the human kappa light chain is preceded by a multiple cloning site containing four restriction sites: AgeI, BstEII, NcoI, and BsiWI. We recommend using the AgeI restriction site for insertion of the 5' end of the variable region including the native signal sequence. If the immunoglobulin signal sequence is unknown, pFUSE2ss and pFUSE2ss plasmids containing a signal sequence should be used. In pFUSE2-CLIg-hk, use BsiWI as the 3' cloning site for the VL in order to preserve the immunoglobulin kappa constant amino acid sequence.

Note:

- Using NcoI as the 3' cloning site will introduce amino acid changes that may not be suitable for some purposes.

- 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 VH, a NheI restriction site must be introduced at the 3' end (for pFUSE-CHIg plasmids featuring the human IgG isotypes; see RELATED PRODUCTS). 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.

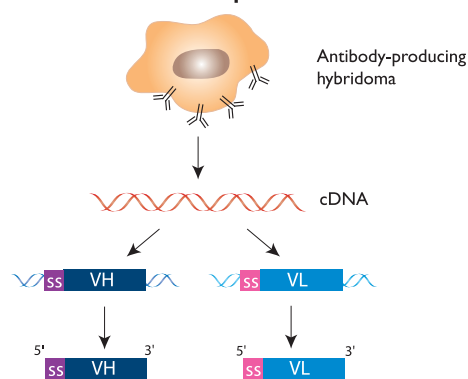
OR
Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

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

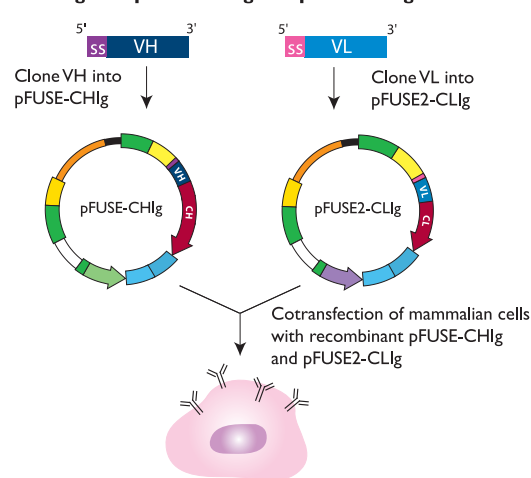
Antibody production can be analyzed by different techniques including SDS-PAGE, flow cytometry, ELISA, or a bioactivity assay.

Antibody generation using pFUSE-CHIg & 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
pFUSE-CHIg-hG1	pfuse-hchg1
pFUSE-CHIg-hG2	pfuse-hchg2
pFUSE-CHIg-hG3	pfuse-hchg3
pFUSE-CHIg-hG4	pfuse-hchg4
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5
Blasticidin	ant-bl-1

TECHNICAL SUPPORT

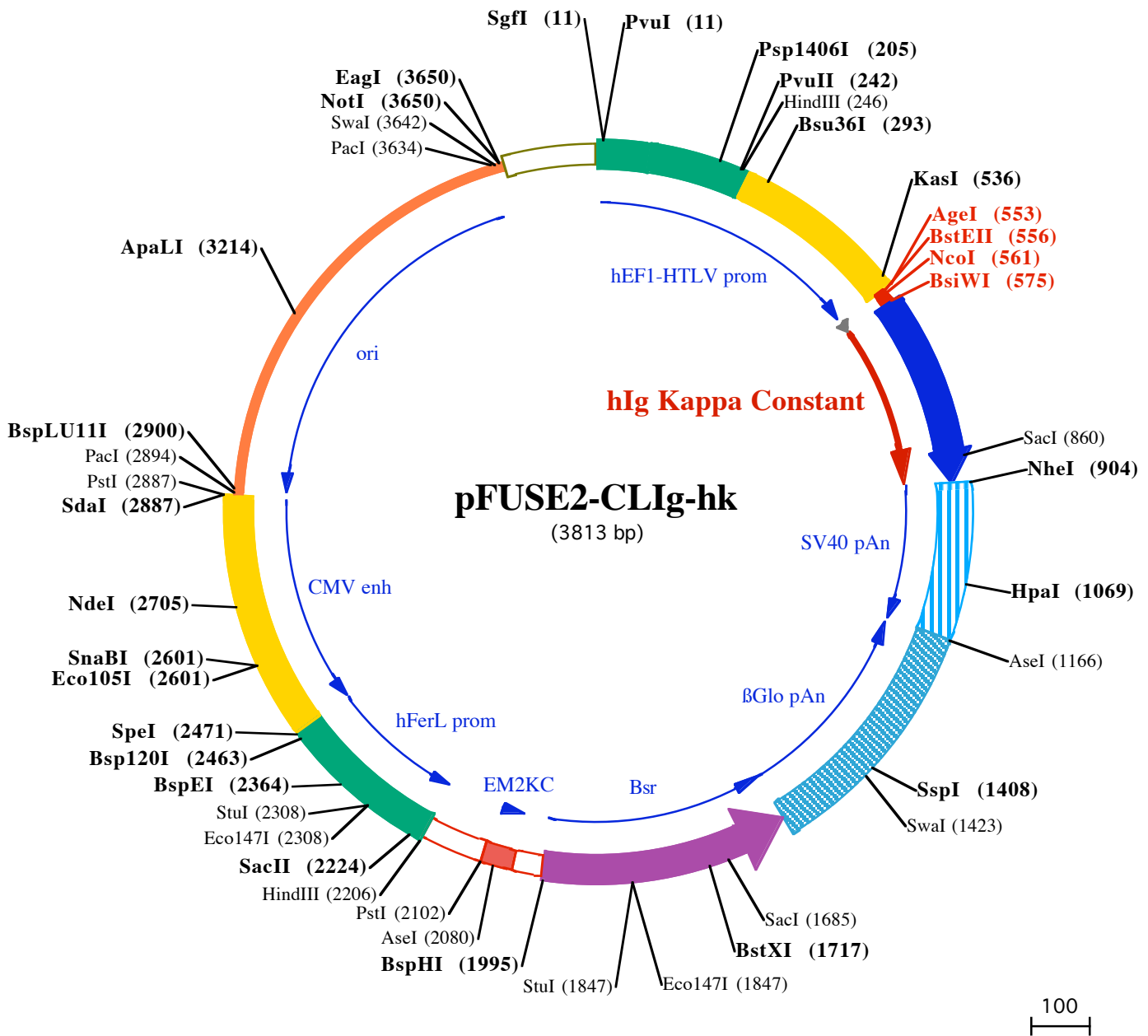
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PvuI (11)
SgfI (11)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGCGAGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA
101 GAGAAGTGGCGCGGGTAAACTGGGAAAGTATGCTGCTGACTGGCTCCGCCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246)
Psp1406I (205) **PvuII (242)** **Bsu36I (293)**
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGGC
301 GCCATCCACGCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCTGAACTGCGTCCGCGCTAGGTAAGTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCGGGCTCTCCACGCTTTGCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCGTTT

NcoI (561)
BstEII (556)
KasI (536) **AgeI (553)** **BsiWI (575)**
501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGGCGCTACCTGAGATCACCGGTACCCATGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTT
601 CATCTTCCCGCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTACAGTGAAG
8▶ I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K
701 GTGGATAACGCCCTCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGACGCAAGGACAGCACCACAGCTCAGCAGCACCCTGACGCTGAGCA
42▶ V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T L S
801 AAGCAGACTACGAGAAACAAAGTCTACGCCTGCGAAGTCAACCCATCAGGGCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGAGAGTGTAGAG
75▶ K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C •
901 GGAGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTAT

NheI (904)
1001 TGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAACAAATGCATTCATTTTATGTTTC

HpaI (1069)
1101 AGGTTACAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTAATTTCAAATAACAGCATAGCAAACTTTAAACC
1201 TCCAAATCAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCT
1301 TCTTTCATGGAGTTAAGATATAGTGATTTTTCCCAAGTTTGAAGTCTTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTT

SspI (1408) **Swal (1423)**
1401 AGTAAAATATTTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCC
1501 CAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTTGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCTGGTGTACTTGAAGGGG
141▶ • N R T Y K L P
1601 ATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGCACATGCCAC
132▶ I L E E I T T K V L K G N M E I L V F C D P A Y D S I L E R C M G C
1701 AGGGGCTGACCACCCTGATGGATCTGTCCACCTCATCAGAGTAGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCCTCTGCCGTTGCTCACAGCAGA
99▶ P S V V R I S R D V E D S Y P H R V A V I T D F D K Q G N S V A S
1801 CCCAATGGCAATGGCTTCCAGCAGACAGTGCACCTGCAATGTAGGCCCTCAATGTGGACAGCAGAGATGATCTCCCAAGTCTTGGTCTGATGGCCGC
66▶ G I A I A E A C V T V R G I Y A E I H V A S I I E G T K T R I A A
1901 CCGACATGGTCTTGTGCTCATAGACATGGTATCTCTCAGTGGCAGCTCCACCAGTCCAGATCCTGCTGAGAGATGTTGAAGTCTTCATGA
32▶ G V H H K N D E Y L M T I K E T A V E V L E L D Q Q S I N F T K M
2001 TGGCTCCTCctgtcaggagaggaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCTTATGATTAATTGTCAAACCTAGGGCTG
AseI (2080) PstI (2102)
2101 CAgggttcagatgtgccacttttctgctgactgccccatctcctgccccctttccaggcatagacagtcagtgacttacAAACTCAGAGGGGAGAA
HindIII (2206) SacII (2224)
2201 GGCAGAAAGCTTGAGACAGACCCGCGGACCGCGAAGTGCAGGGGACGTGGCTAGGGGCGGCTTCTTTTATGGTGCAGCCGCGCTCGGAGGAGGGCGCT
StuI (2308) Eco147I (2308) BspEI (2364)
2301 CGGGGAGGCCATGCGGCAACTGTGCGGTGGCAGGAGCGGGGCGAAGGCCGTGCTGACCAATCCGGAGCAGATAGGAGTCTCAGCCCCCGCCCCAAA

SpeI (2471)
Bsp120I (2463)
2401 GCAAGGGGAAGTACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGGCTTGGGGGCTTGGGGCCTGACTAGTCAAAACAAACTCCATTGACGTCA
SnaBI (2601) Eco105I (2601)
2501 ATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGACTGCCAAAACCGCATCAICATGGTAATAGCGATGACTAATA
2601 CGTAGATGACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGCATAATGCCAGCGGGCCATTTACCGTCAATTGACGTCAATAGGGGGCTACT
2701 TGGCATATGATACTTGTGACTGCAAGTGGGCGTGTACCGTAAATACTCCACCATTGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGA
NdeI (2705)

PacI (2894)

PstI (2887)

SdaI (2887) **BspLU11I (2900)**

2801 ACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAGAA

2901 CATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAT

3001 CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGC

3101 CGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGGTAGGTCGTTGCTC

ApaLI (3214)

3201 CAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG

3301 CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAA

3401 GAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGG

3501 TTTTTTGGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAAC

EagI (3650)

PacI (3634) SmaI (3642) **NotI (3650)**

3601 TCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTGGTTTTT

3701 GTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACA

3801 TTTCTCTATCGAA

Blasticidin

Selection antibiotic; cell culture tested

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

<http://www.invivogen.com/blastcidin>

For research use only

Version 20J13-MM

PRODUCT INFORMATION

Contents

Blasticidin hydrochloride is supplied as a sterile filtered solution at 10 mg/ml in HEPES buffer. It is available in 4 pack sizes:

- ant-bl-05: 5 x 1 ml (50 mg)
- ant-bl-1: 10 x 1 ml (100 mg)
- ant-bl-5: 50 x 1 ml (500 mg)
- ant-bl-5b: 1 x 50 ml (500 mg)

Storage and stability

- Blasticidin 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.

Note: Blasticidin is stable for 2 weeks at room temperature.

QUALITY CONTROL

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

- Purity: ≥95% (HPLC)
- Endotoxin level: < 1 EU/mg
- Physicochemical characterization (pH, appearance)
- Cell culture tested: potency validated in blasticidin-sensitive and blasticidin-resistant mammalian cell lines
- Non-cytotoxicity of trace contaminants: absence of long-term effects confirmed in blasticidin-resistant cells

BACKGROUND

Blasticidin is a selection antibiotic that acts on both eukaryotic and prokaryotic cells. It is a peptidyl nucleoside antibiotic isolated from the culture broth of *Streptomyces griseochromogenes*. It specifically inhibits protein synthesis in both prokaryotes and eukaryotes by inhibiting peptide bond formation in the ribosomal machinery. Three blasticidin resistance genes have been cloned and sequenced: an acetyl transferase gene, *bls* from a blasticidin producer strain¹, and two deaminase genes, *bsr* gene from *Bacillus cereus*², and *BSD* gene from *Aspergillus terreus*³.

Both *bsr* and *BSD* genes are used as dominant selectable markers for gene transfer experiments in mammalian and plant cells. Although blasticidin was developed as a selection agent for mammalian cells, it can also be used in *E. coli*.

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

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

SELECTION CONDITIONS

- *Escherichia coli*

E. coli is poorly sensitive to blasticidin, but transformants resistant to blasticidin can be selected on low salt LB agar medium (pH 8) supplemented with 100 µg/ml blasticidin. High pH enhances the activity of blasticidin.

- Mammalian cells

The working concentration of blasticidin for mammalian cell lines varies from 1 to 10 µg/ml, in a few cases up to 30 µg/ml. In a starting experiment we recommend to determine optimal concentrations of antibiotic required to kill your host cell line. After treatment, cell death occurs rapidly, allowing the selection of transfected cells with plasmids carrying the *bsr* or *BSD* genes in as little as 7 days post-transfection. Suggested concentrations of blasticidin for selection in some examples of mammalian cells are listed below.

Cell line	Medium	Blasticidin conc.	Ref.
CHO (Chinese hamster ovarian cells)	DMEM	5-10 µg/ml	4, 5
HEK293 (Human embryonic kidney cells)	DMEM	5-15 µg/ml	6, 7
HeLa (Human uterine cells)	DMEM	2.5-10 µg/ml	8, 9
Neuro2a (Mouse neuroblasts)	DMEM	30 µg/ml	10
THP-1 (Human monocytes)	RMPI	10 µg/ml	11

1. Perez-Gonzalez J. et al., 1990. Cloning and characterization of the gene encoding a blasticidin S acetyltransferase from *Streptovorticillum* sp. Gene. 86:129-34.
2. Izumi M. et al., 1991. Blasticidin S-resistance gene (*bsr*): A novel selectable marker for mammalian cells. Exp.Cell Res.197:229-33.
3. Kimura M. et al., 1994. Blasticidin S deaminase gene from *Aspergillus terreus* (*BSD*): a new drug resistance gene for transfection of mammalian cells. Biochim. Biophys. Acta. 1219:653-9.
4. Dorgham K. et al., 2009. An engineered CX3CR1 antagonist endowed with anti-inflammatory activity. J Leukoc Biol. 86(4):903-11.
5. LeBon L. et al., 2014. Fringe proteins modulate Notch-ligand cis and trans interactions to specify signalling states. eLife Sci. 3:e02950.
6. Tomecki R. et al., 2014. Multiple myeloma-associated hDIS3 mutations cause perturbations in cellular RNA metabolism and suggest hDIS3 PIN domain as a potential drug target. Nucleic Acids Res. 42:1270-90.
7. Edbauer D. et al., 2004. Co-expression of nicastrin and presenilin rescues a loss of function mutant of APH-1. J Biol Chem. 279:37311-5.
8. Khandelia P. et al., 2011. Streamlined platform for short hairpin RNA interference and transgenesis in cultured mammalian cells. PNAS 108:12799-804.
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10. Matsumoto G. et al., 2011. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. Mol Cell. 44:279-89.
11. Schepetkin IA. et al., 2009. Immunomodulatory activity of oenothin B isolated from *Epilobium angustifolium*. J Immunol. 183:6754-66.

TECHNICAL SUPPORT

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RELATED PRODUCTS

Product	Description	Catalog Code
Other selection antibiotics		
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
Zeocin™	Selection antibiotic for the <i>Sh ble</i> gene	ant-zn-1
Plasmids encoding the <i>bsr</i> gene		
pMOD2-Blast	Plasmid encoding a synthetic <i>bsr</i> gene	pmod2-blast
pSELECT-blasti-LacZ	LacZ-expression plasmid selectable with blasticidin	psetb-lacz
pSELECT-blasti-mcs	Expression plasmid selectable with blasticidin	psetb-mcs
pUNO1-bsr	Expression plasmid selectable with blasticidin	puno1-bsr

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