

pFUSE2-CLIg-hI2

Plasmid featuring the constant region of the human immunoglobulin lambda 2 light chain

Catalog # pfuse2-hcll2

For research use only

Version # 08F19-SV

PRODUCT INFORMATION

Content:

- 20 µg of pFUSE2-CLIg-hI2 plasmid provided as lyophilized DNA.
- 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar)

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 *E. coli* Fast-Media® Blas at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pFUSE-CLIg plasmids feature the kappa light chain constant region of human or mouse immunoglobulin.

The CL region is preceded by a multiple cloning site to facilitate the insertion of the variable region (VL) of a given antibody. Coexpression of the pFUSE-CLIg cloned with the VL region with a pFUSE-CHIg cloned with the variable heavy (VH) region will allow for the expression of recombinant antibodies. With the large choice of CH IgG isotypes available it is possible to switch the isotype of a given antibody in order to create antibodies with the same antigen affinity but with different effector functions.

Since the pFUSE-CLIg and pFUSE-CHIg plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of pFUSE-CLIg and pFUSE-CHIg plasmids.

IgG antibodies produced using pFUSE-CLIg and pFUSE-CHIg plasmids can be purified by protein A or protein G affinity chromatography. They can also be purified using immobilized protein L agarose (see related products).

PLASMID FEATURES

• **human IgL2C (Ig Lambda 2 Light constant domain):** When cloning your VL (lambda J segment) of choice in the MCS, care must be taken to preserve the reading frame.

Note: Using *Avr* II as the 3' cloning site for the lambda J segment will preserve the standard immunoglobulin lambda 2 constant amino acid sequence (VL. . .) and will allow you to clone exactly the variable lambda of your choice. Using *Acc*65 I as the 3' cloning site will work for any variable regions that end with the amino acids GTKLTVL.

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1 α (EF-1 α) core promoter² and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat³. The EF-1 α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 α 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 mRNA⁴.

• **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.

• **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*.

• **β Glo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁵.

TECHNICAL SUPPORT

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METHODS

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 H₂O. Store resuspended plasmid at -20°C.

Selection of bacteria with *E. coli* Fast-Media®

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic. Fast-Media® Blas is available separately: #fas-bl-l (liquid), #fas-bl-s (agar).

- 1- Pour the contents of a Fast-Media® pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

References:

1. Nott A, et al. 2003. A quantitative analysis of intron effects on mammalian gene expression. RNA. 9(5):607-17.
2. Kim DW et al. 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23.
3. 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.
4. 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.
5. 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.

RELATED PRODUCTS

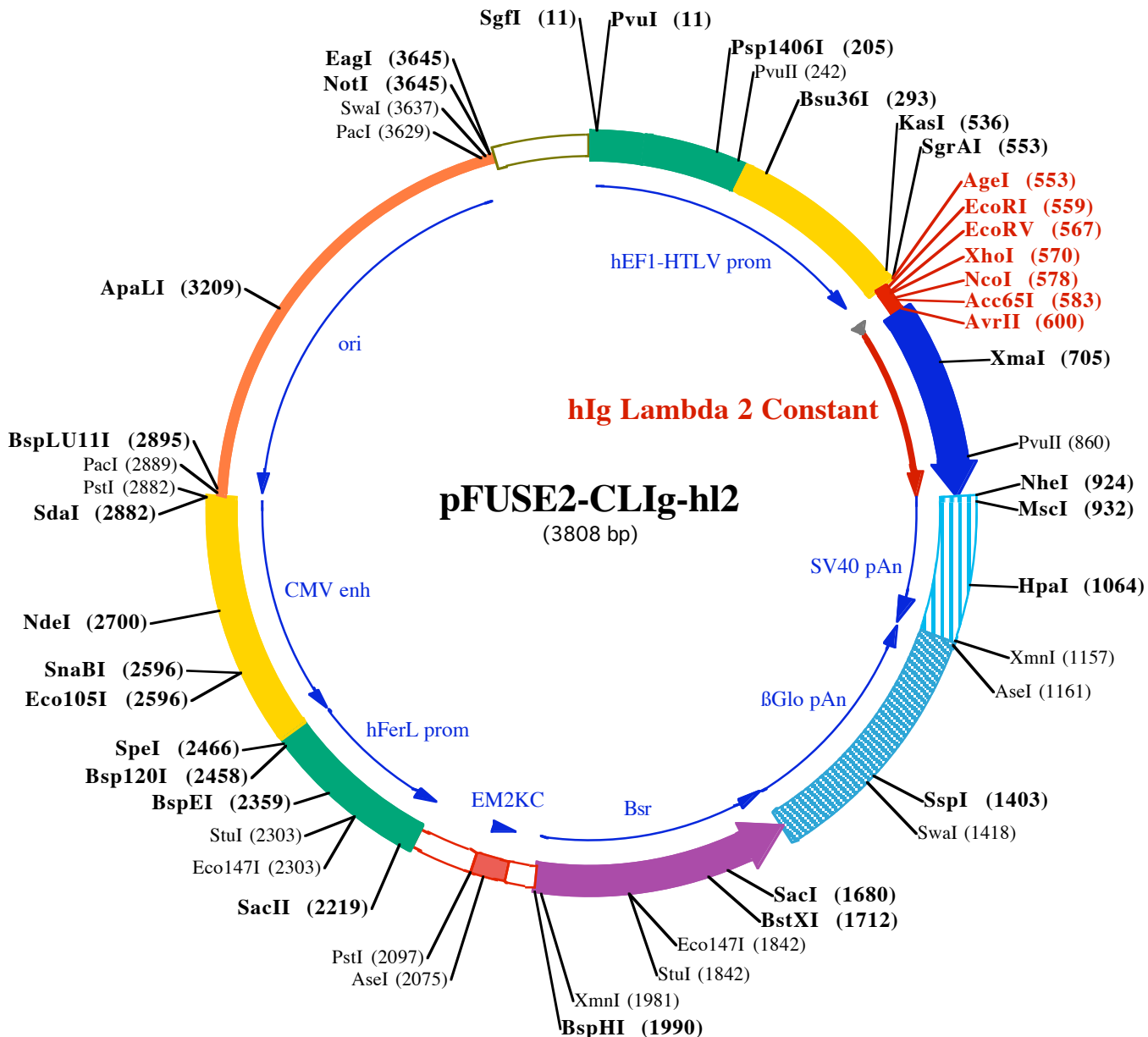
Product	Catalog Code
pFUSE-CHiG-hG1	pfuse-hchg1
pFUSE-CHiG-hG2	pfuse-hchg2
pFUSE-CHiG-hG3	pfuse-hchg3
pFUSE-CHiG-hG4	pfuse-hchg4
Protein L / Agarose	gel-protl-2
Blasticidin	ant-bl-1
Fast-Media® Blas TB	fas-bl-l
Fast-Media® Blas Agar	fas-bl-s

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100

PvuI (11)
SgfI (11)
1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGGCAGAGCCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTCCGCAATTGAACGGGTGCCTA
101 GAGAAGTGGCGGGGTAAACTGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

Psp1406I (205) **PvuII (242)** **Bsu361 (293)**
201 GTGAACGTTCTTTTTCGAACGGTTTCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTAGGACC
301 GCCATCCACGCGGTTGAGTGCCTCTGCCGCTCCCGCTGTGGTGCCTCCTGAATCGCTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC
401 GGGCCTTTGTCGGCGCTCCCTTGAGCCTACCTAGACTCAGCGGCTCTCCACGCTTTGCTGACCCTGCTTGTCTCAACTCTACGCTTTTGTTCGTTT

EcoRI (559)
KasI (536) **AgeI (553)** **SgrAI (553)** **EcoRV (567)** **NeoI (578)** **XhoI (570)** **Acc65I (583)** **AvrII (600)**
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGGCCCTACCTAGATCACCGGTGAATTCGATATCTCGAGCACCATGGGTACCAAGCTTACCGTCT
601 CTAGGTCAGCCCAAGGCTGCCCTCGTTCACCTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAAACAGGCCACACTGGTGTGCTCATAAGTACT
701 TCTACCCGGGAGCGTGACAGTGGCCGGAAGGCAGATAGCAGCCCGTCAAGGCGGAGTGGAGACCACACCCTCCAAACAAAGCAACAAAGTAA
801 CGCGCCAGCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCACAGAAGCTACAGCTGCCAGGTACGATGAAGGGAGCACCCTGGGAGAGACA
901 GTGGCCCTACAGAATGTTATAGTCTAGCTGGCCAGACATGATAAGATCATTGATGAGTTGGACAACCAACAATGACAGTGAAGGAAATGCTT
100 V A P T E C S •

XmaI (705) **PvuII (860)**
701 TCTACCCGGGAGCGTGACAGTGGCCGGAAGGCAGATAGCAGCCCGTCAAGGCGGAGTGGAGACCACACCCTCCAAACAAAGCAACAAAGTAA
801 CGCGCCAGCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCACAGAAGCTACAGCTGCCAGGTACGATGAAGGGAGCACCCTGGGAGAGACA
901 GTGGCCCTACAGAATGTTATAGTCTAGCTGGCCAGACATGATAAGATCATTGATGAGTTGGACAACCAACAATGACAGTGAAGGAAATGCTT
100 V A P T E C S •

MscI (932) **NheI (924)**
901 GTGGCCCTACAGAATGTTATAGTCTAGCTGGCCAGACATGATAAGATCATTGATGAGTTGGACAACCAACAATGACAGTGAAGGAAATGCTT
100 V A P T E C S •

HpaI (1064)
1001 TATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGTGAATAAACAAAGTTAACAAACAATGACATTCATTTTATGTTTCAGGTT

AseI (1161) **XmnI (1157)**
1101 CAGGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATCTAAAATACAGCATAGCAAACTTTAACCTCCAA
1201 ATCAAGCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTT
1301 CATGGAGTTAAGATATAGTGTATTTTCCAAGTTTGAACAGTCTTTCATTTCTTTATGTTTTAATGCACTGACCTCCACATTCCCTTTTAGTAA

SspI (1403) **Swal (1418)**
1401 AATATTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTT
1501 TAGTAGTTGGACTTAGGGAACAAGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCTGGTGTACTTGAGGGGGATGAG
1601 TTCCTCAATGGTGGTTTTGACCAGCTTGCATTATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTGCACATGCCACAGGGG
1701 CTGACCACCTGATGGATCTGCCACCTCATCAGAGTAGGGTGCCTGACAGCCACAATGGTGTCAAAGTCTTCTGCCGCTTGTCTACAGCAGACCCAA
1801 TGGCAATGGCTTCAGCAGACAGTACCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCGAGTCTTGGTCTGATGGCCGCCCGAC
1901 ATGGTGTCTTGTCTCATAGAGCATGGTGTCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGCTTTCATGATGGCT
2001 CCTCctgcaggagaggaagagaagaaggttagtacaattgCTATAGTGTGATTATACTATGCTTATGATTAATTGTCAAACCTAGGGCTCAGggg
2101 ttcatagtgccacttttctgcactgccccatctcctgccaccctttccaggcatagacagtcagtgacttacCAAACCTCACAGGAGGAGAAGGCAG
2201 AAGCTTGAGACAGACCCGGGACCCGAACCTGCGAGGGGACGTGGCTAGGGCGCTTCTTTTATGGTGCAGCCGCCCTCGGAGGCAGGGCGCTCGGGG
2301 AGGCCTAGCGCAATCTGCGGTGGCAGGAGGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAG

StuI (2303) **Eco147I (2303)** **BspEI (2359)**
2301 AGGCCTAGCGCAATCTGCGGTGGCAGGAGGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCGCCCAAGCAAG

SpeI (2466) **Bsp120I (2458)**
2401 GGGAAAGTACGCGCTGTAGCGCCAGCGTGTGTAAGTGGGGGCTTGGGGGGTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCATGGG

SnaBI (2596) **Eco105I (2596)**
2501 GTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAG

NdeI (2700)
2601 ATGTAAGTCCAAAGTAGGAAAGTCCATAAGGTCAATGTAAGTGGGCATAATGCCAGGCGGGCATTACCCTGATTGACGTCAAATAGGGGGCTACTTGGCA
2701 TATGATACACTTGTACTGCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAAATGGAAGTCCCTATTGGCGTTACTATGGGAACATA

Fast-Media®

Microwaveable media for selection and propagation of *E. coli* transformants

Catalog # fas-xx-l, fas-xx-s, fas-xx-xgal

For research use only

Version # 09G27-MM

PRODUCT INFORMATION

Contents:

E. coli **Fast-Media**® are prepared as individual sealed pouches containing the necessary amount of powder for preparation of 200 ml of selective liquid or agar medium.

30 pouches are supplied for each order of TB or Agar and 20 pouches are supplied for each order of XGal Agar.

Storage and stability:

Fast-Media® are shipped at room temperature, and must be stored in a dry and cool place. They are stable for at least one year at room temperature.

When properly prepared, **Fast-Media**® plates or broths are stable several weeks at 4°C, and remain sterile and selective.

Quality control:

The high quality and performance of each formulation has been tested with some widely used and proprietary *E. coli* K12 derived strains*. These include DH5α, Top10, MC1061, XL1 blue, JM 109, TB1, GT100, GT110, GT115, GT116.

The adequate plasmids carrying the appropriate *E. coli* resistance genes are used as positive control.

**E. coli* recipient strains carrying the Tn5 transposon are resistant to Kanamycin and Zeocin™.

GENERAL PRODUCT USE

E. coli **Fast-Media**® are microwaveable ready-to-use solid or liquid media, supplied with a selective antibiotic, and chromogenic substrates (for five references), therefore designed for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies.

- **Fast-Media**® Agar formulation is LB based agar medium supplemented with selective antibiotic, it is used for selection of resistant *E. coli* colonies after transformation by vectors carrying a selection resistance gene.

- **Fast-Media**® X-Gal formulation is a LB based agar medium supplemented with selective antibiotic, X-Gal and IPTG. It is used for detection of blue/white resistant colonies after transformation by a vector carrying *LacZ* gene.

- **Fast-Media**® TB formulation is a Terrific Broth based liquid medium supplemented with selective antibiotic. It's used for high cell density culture of transformed bacteria, and extraction of high quantity and quality of required plasmid.

FAST-MEDIA® FEATURES

E. coli **Fast-Media**® offer researchers a quick and convenient way to prepare 200 ml of liquid culture medium, or 8-10 agar plates in about five minutes USING A MICROWAVE INSTEAD OF AN AUTOCLAVE.

E. coli **Fast-Media**® are available with a large variety of prokaryotic selective agents including Ampicillin, Blasticidin S, Hygromycin B, Kanamycin, Puromycin and Zeocin™ (see table below). **Fast-Media**® is also available with no selective agent (Base) that can be prepared with or without antibiotics.

	Agar	X-Gal	TB
Base	√		√
Ampicillin	√	√	√
Blasticidin	√	√	√
Hygromycin	√	√	√
Kanamycin	√	√	√
Puromycin	√		√
Zeocin™	√	√	√

SPECIAL HANDLING

Caution should be exercised during handling of **Fast-Media**® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breath the dust.

METHOD

For customer convenience, procedure is directly printed on each pouch.

- 1- Pour the pouch contents into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled or deionized water.
- 3- Mix thoroughly by swirling the glass bottle or flask.
- 4- Heat in a microwave oven on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes).

Do not heat in a closed container.

5- Swirl gently to mix the preparation and re-heat for 30 seconds. Swirl gently again.

6- Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.

7- Allow the medium to cool to 50-55 °C, use directly for liquid medium, or pour plates for solid medium.

Caution: Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.

Note: Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.

For preparation of supplemented Fast-Media® Base.

- Follow the instructions above and when media has cooled to 50-55 °C add the antibiotic at the appropriate concentration for selection of *E. coli*.

TECHNICAL SUPPORT

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