pFUSE-hIgG4e1-Fc1

Plasmid containing a human engineered IgG4 Fc region

Catalog # pfc1-hg4e1

For research use only

Version # 11K13-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE-hIgG4e1-Fc1** plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Zeo (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® Zeo 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-Fc is a family of plasmid developed to facilitate the construction of Fc-fusion proteins by fusing the effector region of a protein to the Fc region of an immunoglobulin G (IgG).

pFUSE-Fc plasmids yield high levels of Fc-fusion proteins. The level of expression is usually in the µg/mL range. They can be transfected in a variety of mammalian cells, including myeloma cell lines, CHO cells, monkey COS cells and human embryonic kidney (HEK)293 cells, cells that are commonly used in protein purification systems.

pFUSE-Fc plasmids allow the secretion of Fc-Fusion proteins. As Fc-Fusion proteins are secreted, they can be easily detected in the supernatant of pFUSE-Fc-transfected cells by SDS-PAGE. Furthermore, functional domains can be identified by immunoblotting and ligand blotting.

Fc-Fusion proteins can be easily purified by single-step protein A affinity chromatography.

InvivoGen provides pFUSE-Fc vectors featuring Fc regions from different species and isotypes. In humans, there are four isotypes: IgG1, IgG2, IgG3 and IgG4. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). IgG isoforms exert different levels of effector functions increasing in the order of IgG4<IgG2<IgG1≤IgG3. Human IgG1 dispays high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells.

Under certain circumstances, for example when depletion of the target cell is undesirable, abrogating effector functions is required. On the contrary, in the case of antibodies intended for oncology use, increasing effector functions may improve their therapeutic activity[1]. Modifying effector functions can be achieved by engineering the Fc regions to either improve or reduce their binding to FcγRs or the complement factors. Amino acids substitutions have been made in the human IgG1 Fc region in order to increase or reduce its ADCC and CDC.

PLASMID FEATURES

• **hIgG4e1-Fc (human IgG4 engineered Fc):** The Fc region comprises the CH2 and CH3 domains of the IgG heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc-Fusion protein, allowing each part of the molecule to function independently.
The Fc mutation S228P has been reported to reduce Fab-arm exchange[2]. pFUSE-hIgG4e1-Fc1 contains the S228P mutation.

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[3] 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[4]. 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[5].

• **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.

• **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*.

• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[6].

1. Carter PJ., 2006. Potent antibody therapeutics by design. Nat Rev Immunol. 6(5):343-57.
2. Labrijn AF. *et al.*, 2009. Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 *in vivo*. Nat Biotechnol. 27(8):767-71.
3. 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.
4. 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.
5. 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.
6. 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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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 H_2O. 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® Zeo is available separately: #fas-zn-l (liquid), #fas-zn-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.

RELATED PRODUCTS

Product	Catalog Code
Zeocin™	ant-zn-1
Fast-Media® Zeo TB	fas-zn-l
Fast-Media® Zeo Agar	fas-zn-s

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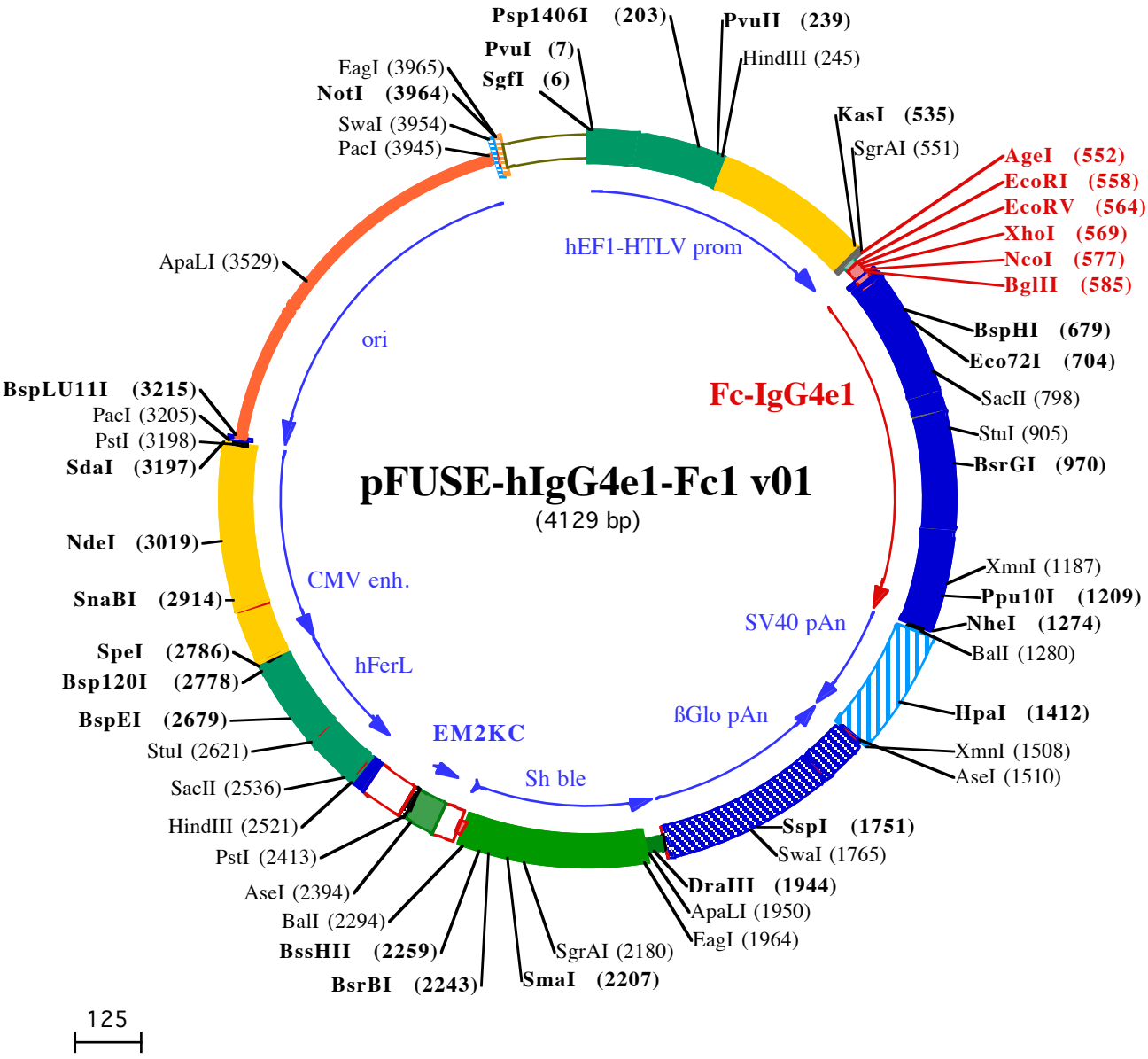
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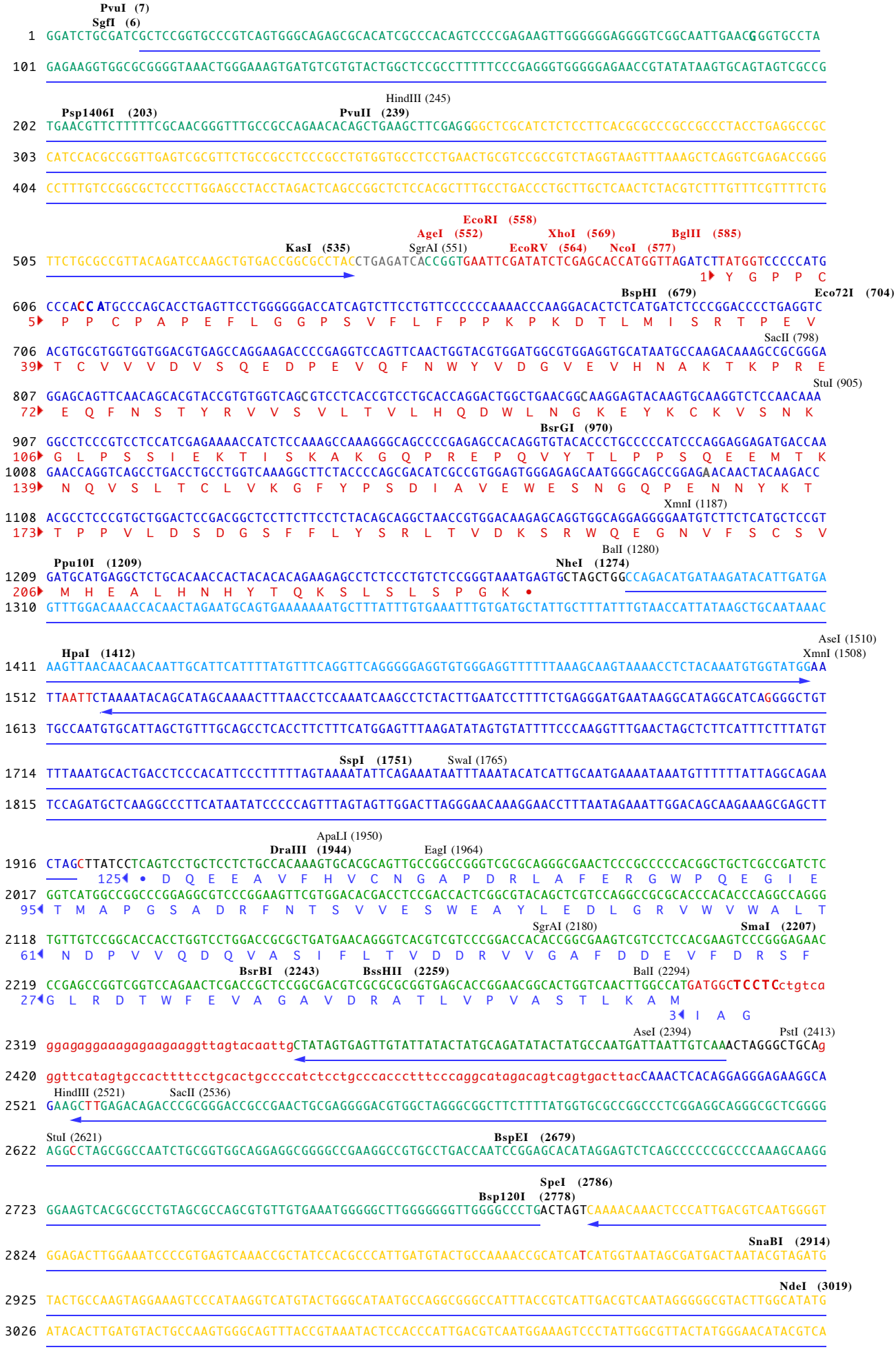
Fc-IgG4e1
hEF1-HTLV prom
ori
CMV enh.
hFerL
EM2KC
Sh ble
ßGlo pAn
SV40 pAn
Psp1406I (203)
PvuII (239)
PvuI (7)
HindIII (245)
SgfI (6)
EagI (3965)
NotI (3964)
SwaI (3954)
PacI (3945)
KasI (535)
SgrAI (551)
AgeI (552)
EcoRI (558)
EcoRV (564)
XhoI (569)
NcoI (577)
BglII (585)
ApaLI (3529)
BspHI (679)
Eco72I (704)
SacII (798)
StuI (905)
BsrGI (970)
BspLU11I (3215)
PacI (3205)
PstI (3198)
SdaI (3197)
NdeI (3019)
XmnI (1187)
Ppu10I (1209)
NheI (1274)
SnaBI (2914)
BalI (1280)
SpeI (2786)
Bsp120I (2778)
HpaI (1412)
BspEI (2679)
XmnI (1508)
StuI (2621)
AseI (1510)
SacII (2536)
SspI (1751)
HindIII (2521)
SwaI (1765)
PstI (2413)
DraIII (1944)
AseI (2394)
ApaLI (1950)
BalI (2294)
EagI (1964)
BssHII (2259)
SgrAI (2180)
BsrBI (2243)
SmaI (2207)
125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SgfI (6)
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101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCG
HindIII (245)
Psp1406I (203)
PvuII (239)
202 TGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGC
303 CATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGG
404 CCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTG
EcoRI (558)
AgeI (552)
XhoI (569)
BglII (585)
KasI (535)
SgrAI (551)
EcoRV (564)
NcoI (577)
505 TTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTGAATTCGATATCTCGAGCACCATGGTTAGATCTTATGGTCCCCCATG
1 Y G P P C
BspHI (679)
Eco72I (704)
606 CCCACCATGCCCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTC
5 P P C P A P E F 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
SacII (798)
706 ACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGA
39 T C V V V D V S Q E D P E V Q F N W Y V D G V E V H N A K T K P R E
StuI (905)
807 GGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA
72 E Q F N S T Y 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
BsrGI (970)
907 GGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAA
106 G L P S S I E K T I S K A K G Q P R E P Q V Y T L P P S Q E E M T K
1008 GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
139 N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T
XmnI (1187)
1108 ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGT
173 T P P V L D S D G S F F L Y S R L T V D K S R W Q E G N V F S C S V
BalI (1280)
Ppu10I (1209)
NheI (1274)
1209 GATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCTAGCTGGCCAGACATGATAAGATACATTGATGA
206 M H E A L H N H Y T Q K S L S L S P G K •
1310 GTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAC
AseI (1510)
HpaI (1412)
XmnI (1508)
1411 AAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAA
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SspI (1751)
SwaI (1765)
1714 TTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAA
1815 TCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTT
ApaLI (1950)
DraIII (1944)
EagI (1964)
1916 CTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTC
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 E G I E
2017 GGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGG
95 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 V W A L T
SgrAI (2180)
SmaI (2207)
2118 TGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAAC
61 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 D R S F
BsrBI (2243)
BssHII (2259)
BalI (2294)
2219 CCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtca
27 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
3 I A G
AseI (2394)
PstI (2413)
2319 ggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAg
2420 ggttcatagtgccacttttcctgcactgccccatctcctgcccacccctttcccaggcatagacagtcagtgacttacCAAACTCACAGGAGGGAGAAGGCA
HindIII (2521)
SacII (2536)
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StuI (2621)
BspEI (2679)
2622 AGGCCTAGCGGCCAATCTGCGGTGGCAGGAGGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCCCCCCGCCCCAAAGCAAGG
SpeI (2786)
Bsp120I (2778)
2723 GGAAGTCACGCGCCTGTAGCGCCAGCGTGTTGTGAAATGGGGGCTTGGGGGGGTTGGGGCCCTGACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGT
SnaBI (2914)
2824 GGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATG
NdeI (3019)
2925 TACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATG
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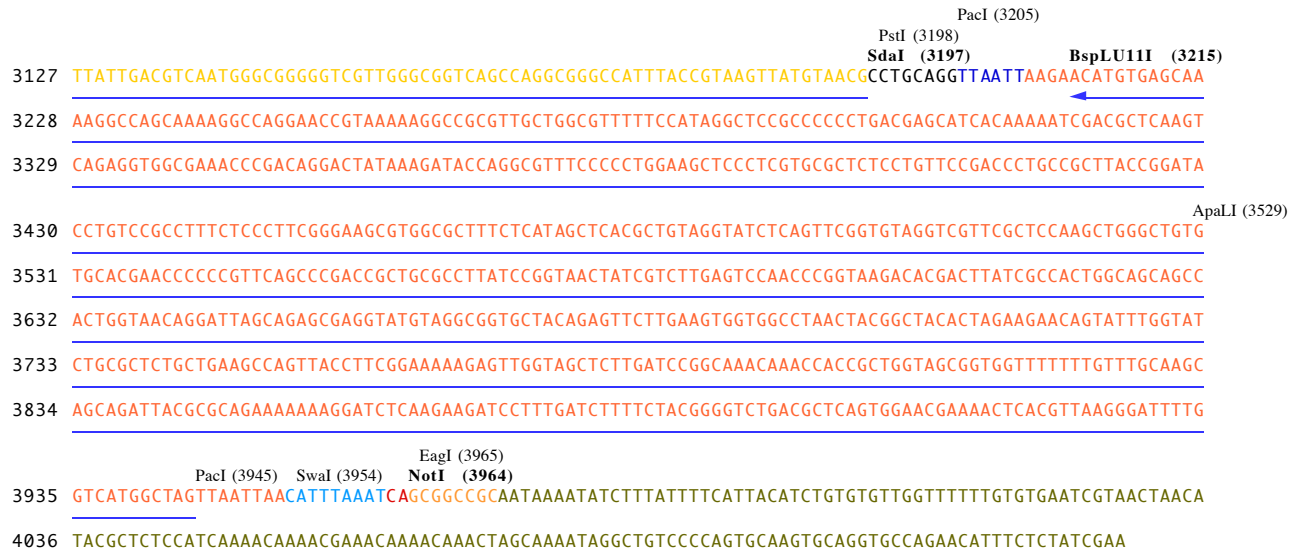
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BspLU11I (3215)
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3329 CAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATA
ApaLI (3529)
3430 CCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG
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EagI (3965)
PacI (3945)
SwaI (3954)
NotI (3964)
3935 GTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACA
4036 TACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA

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 # 10G07-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 2 years at room temperature.

When properly prepared, **Fast-Media®** plates or broths are stable for 4 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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