

# pFUSE-CHlg-mG1

Plasmid featuring the constant region of the mouse IgG1 heavy chain

Catalog # pfuse-mchg1

For research use only

Version # 11C03-MM

## PRODUCT INFORMATION

### Content:

- 20  $\mu$ g of pFUSE-CHlg-mG1 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.

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

## GENERAL PRODUCT USE

pFUSE-CLlg and pFUSE-CHlg 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 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 pFUSE-CLlg and pFUSE2-CHlg plasmids

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1 $\alpha$  (EF-1 $\alpha$ ) 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-1 $\alpha$  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 $\alpha$  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.
- **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.
- **$\beta$ Glo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription<sup>4</sup>.

## pFUSE-CHlg-mG1 specific features

- **Mouse IgHG1 (IgG1 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 pFUSE-CHIg and pFUSE2-CLIg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSE-CHIg-mG1, the constant region of the mouse IgG1 heavy chain is preceded by a multiple cloning site containing six restriction sites: AgeI, EcoRI, EcoRV, XhoI, NheI and Eco47III. 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-CHIg-mG1, use Eco47III (blunt-end cloning) as the 3' cloning site for the VH in order 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 BstAPI (mouse kappa; pFUSE2-CLIg-mk), or AvrII (mouse lambda; pFUSE2-CLIg-ml1 or pFUSE2-CLIg-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.*

### Choice of strategies for the transfection

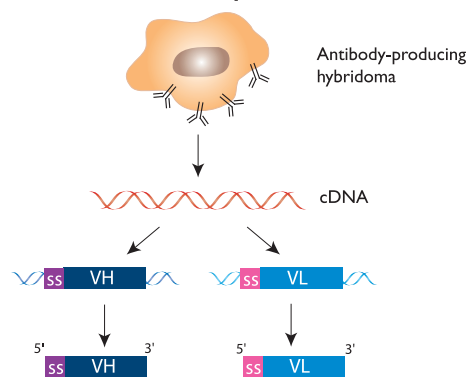
Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

OR

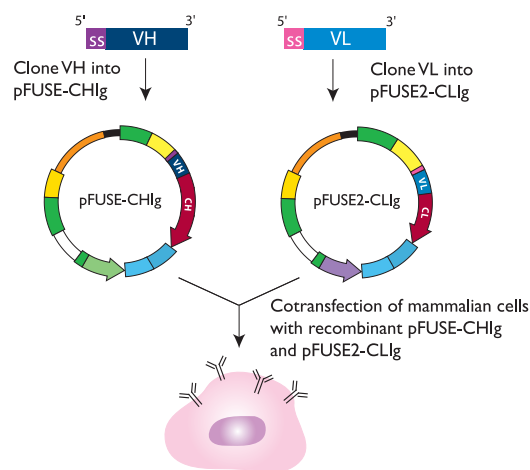
A cotransfection can be performed with the plasmid coding for the light chain and the plasmid coding for the heavy chain. Since the pFUSE2-CLIg and pFUSE-CHIg plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities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

## Antibody generation using pFUSE-CHIg & pFUSE-CLIg

### 1- Obtention of VH and VL sequences



### 2- Cloning into pFUSE-CHIg and pFUSE2-CLIg



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

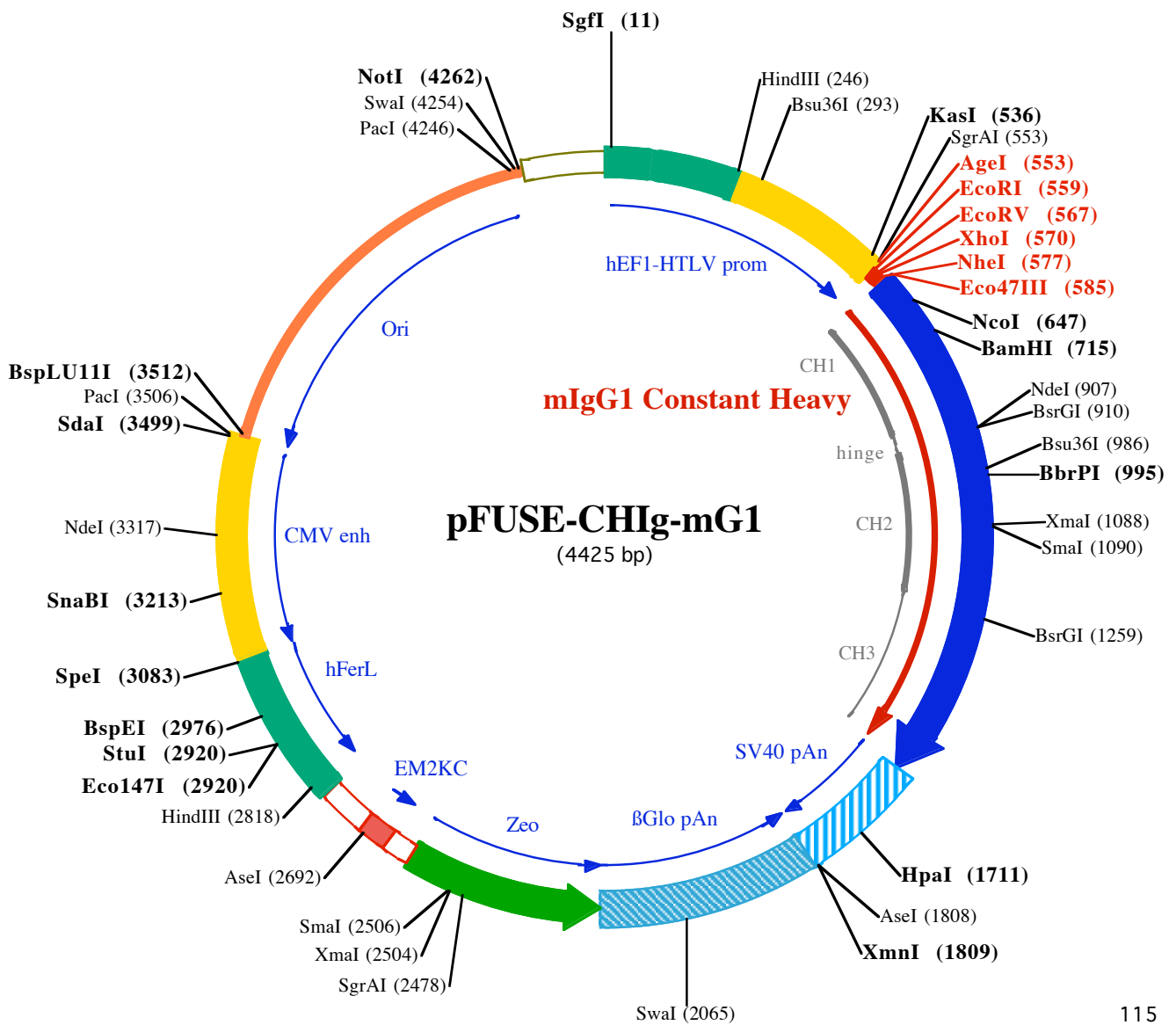
Product	Catalog Code
pFUSE2-CLIg-mk	pfuse2-mclk
pFUSE2-CLIg-ml1	pfuse2-mcll1
pFUSE2-CLIg-ml2	pfuse2-mcll2
pFUSE-CHIg-hG2a	pfuse-mchg2a
pFUSE-CHIg-hG2b	pfuse-mchg2b
pFUSE-CHIg-hG3	pfuse-mchg3
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5
Zeocin™	ant-zn-1
Fast-Media® Zeo TB	fas-zn-1
Fast-Media® Zeo Agar	fas-zn-s

### TECHNICAL SUPPORT

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SgfI (11)  
1 GGATCTGCGATCGCTCCGGTCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGTTCGCAATTGAACGGGTGCCTA  
101 GAGAAGGTGGCGGGGTAACCTGGGAAAGTGATGTCGTGTACTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

HindIII (246) Bsu36I (293)  
201 GTGAACGTTCTTTTCGCAACGGGTTGCCGCCAGAACACAGCTGAAGCTTCAGAGGGCTCGCATCTCTCCTTACGCGCCGCCCTACCTGAGGGC  
301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAATCGCTCCGCGTCTAGTAAGTTAAAGCTCAGGTCGAGACC  
401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCCACGCTTTGCTGACCTGCTTGTCAACTCTACGCTTTGTTTCGTTT

EcoRI (559) XhoI (570) Eco47III (585)  
KasI (536) AgeI (553) SgrAI (553) EcoRV (567) NheI (577)  
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGGGCGCTACCTGAGATCACCGGTGAATTCGATATCTCGAGTGTAGCAGCGCTAAAACGACACCC  
1 A K T T P

NcoI (647)  
601 CCATCTGTCTATCCACTGGCCCTGGATCTGCTGCCAACTAACCATGGTACCTGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGCACG  
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

BamHI (715)  
701 TGACCTGGAAGTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCAGCTGTCTGCACTGTACCTCTACACTCTGAGCAGCTCAGTACTGTCCCTC  
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 CAGCACCTGGCCAGCGAGACCGTCACTGCAACGTTGCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGATTGTGGTTGTAAG  
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

BsrGI (910) NdeI (907) BbrPI (995) Bsu36I (986)  
901 CTTGTCATATGTACAGTCCAGAAAGTATCATCTGTCTTCTTCCCTCCCAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTG  
106 P C I C T V P E V S S V F I F P P K P K D V L T I T L T P K V T C

XmaI (1088) SmaI (1090)  
1001 TTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGTGCACACAGCTCAGACGCAACCCGGGAGGAGCA  
139 V V V D 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 GTTCAACAGCACTTTCCGCTCAGTCAGTGAATTCATCATGACACAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTCAACAGTGCAGCTTTC  
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

BsrGI (1259)  
1201 CTTGCCCCATCGAGAAAACCTCTCCAAAACCAAGGCAGCCGAGGCTCCACAGGTGTACACATTCCACCTCCCAAGGAGCAGATGGCCAAGGATA  
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 AAGTCAGTCTGACCTGCATGATAACAGACTTCTCCCTGAAGACATTACTGTGGAGTGGCAGTGAATGGCAGCCAGCGGAGAACTACAAGAACACTCA  
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 GCCCATCATGGACACAGATGGCTTCTACTTCGTCTACAGCAAGCTCAATGTGAGAAGAGCAACTGGGAGGCAGGAAAATCTTTCACCTGCTCTGTGTTA  
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 CATGAGGGCCTGCACAACCACCTACTGAGAAGAGCCTCTCCACTCTCTGGTAAATGATCCCAAGTGTCCCTAGCTGGCCAGACATGATAAGATACATT  
306 H E G L H N H H T E K S L S H S P G K •  
1601 GATGAGTTGGACAACCAACTAGAATGCAGTGAAGAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGTGC

HpaI (1711)  
1701 ATAAACAAGTTAAACAACAACAAATGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGG

AseI (1808) XmnI (1809)  
1801 TATGGAATTAATTCTAAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCA  
1901 GGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTACCTTCTTTCATGGAGTTTAAAGATATAGTGATTTTCCCAAGGTTTGAACAGCTCTTCATT

SwaI (2065)  
2001 TCTTTATGTTTTAAATGCACTGACCTCCACATTTCCCTTTTATGTAATAATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTA  
2101 TTAGGCAGAATCCAGATGCTCAAGGCCCTTATAATATCCCCAGTTTGTAGTGTGGACTTAGGGAACAAGGAACCTTTAATAGAAATTTGGACAGCAAG  
2201 AAAGCGAGCTTCTAGCTTATCTCAGTCTGCTCCTGTCACAAAGTGCACGAGTTCGCGGGCGGTTCGCGAGGGCGAACTCCCGCCCCACGGCTG  
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 CTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAAGTTCGTGGACACGACCTCCGACCTCGGCGTACAGCTCGCCAGGCCGACCCAC  
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 ACCGAGCCAGGGTGTGTCCGGCACCACCTGGTCTGGACCGCGCTGATGAACAGGGTCACTGCTCCCGGACCACCCGGCGAAGTCTCTCCACGA  
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  
XmaI (2504) SmaI (2506)  
2501 AGTCCCGGAGAACCCGAGCGGTGGTCCGAACTCGACCGTCCGGCGAGCTCGCGCGGTGAGCACCGAACGGCACTGGTCAACTTGGCCATGAT  
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 GGCTCCTCctgtcaggagaggaagagaagaggttagtacaattgCTATAGTGAAGTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTC  
AseI (2692)

2701 AACTAGGGCTGCAgggttcatagtgccacttttctgcactgccccatctctgccaccctttcccaggcatagacagtcagtgacttacCAAActCA  
HindIII (2818)  
2801 CAGGAGGGAGAAGGCAGAAGCTTGAGACAGACCCGCGGACC GCCAACTGCGAGGGGACGTGGCTAGGGCGGCTTCTTTTATGGTGC GCCGCCCTCGG  
StuI (2920)  
Eco147I (2920) BspEI (2976)  
2901 AGGCAGGGCGCTCGGGAGGCC TAGCGGCAATCTGCGGTGGCAGGAGCGGGGCCGAAGGCCGTGCCTGACCAATCCGGAGCACATAGGAGTCTCAGCC  
SpeI (3083)  
3001 CCCC GCCCAAAGCAAGGGGAAGTCACGCGCCTGTAGCGCCAGCGTGTGTGAAATGGGGCTTGGGGGGTTGGGGCCCTGACTAGTCAAAA CAAACTC  
3101 CCATTGACGTCAATGGGGTGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAG  
SnaBI (3213)  
3201 CGATGACTAATACGTAGATGACTGCCAAGTAGGAAAGTCCATAAAGTCATGTACTGGGCATAATGCCAGCGGGCCATTTACCGTCATTGACGTCAAT  
NdeI (3317)  
3301 AGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCACTTTACCGTAAATACTCCACCATTGACGTCAATGGAAAGTCCCTATTGGC  
SdaI (3499)  
3401 GTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTGACGAGCGGGCCATTTACCGTAAGTTATGTAACGCCCTGCAG  
PacI (3506) BspLU11I (3512)  
3501 GTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAG  
3601 CATCACAAAATCGAGCTCAAGTCAGAGGTGGGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAAGTCCCTCGTGGCTCTCCTG  
3701 TTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTA  
3801 GGTGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTAGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGA  
3901 CACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACG  
4001 GCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAGAGTTGGTAGCTTTGATCCGGCAAAACAAACCACCGC  
4101 TGGTAGCGGTGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAG  
PacI (4246) SmaI (4254) NotI (4262)  
4201 TGGAACGAAAACACGTTAAGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTG  
4301 TGTGGTTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAAACAAACGAAACAAACAAACTAGCAAATAGGCTGTCCCCAGTGCAAGTGCA  
4401 GGTGCCAGAACATTTCTCTATCGAA

# 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, Blastidicin 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	√	√	√
Blastidicin	√	√	√
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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