pFUSE-CHIg-hG1e3

Plasmid featuring a mutated constant region of the human IgG1 heavy chain
Catalog # pfuse-hchg1e3
For research use only
Version # 11G04-MM

PRODUCT INFORMATION

Content:
- 20 μg of pFUSE-CHIg-hG1e3 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
- pFUSE-2-CLIg plasmid that features the constant region of the kappa or lambda light chains. pFUSE2-CLlIg plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).
- pFUSE-CHIg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin™.

GENERAL PRODUCT USE

pFUSE-CHIg and pFUSE2-CLlIg 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. 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-CLlIg 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. InvivoGen provides engineered pFUSE-CHIg which express mutant forms of the constant regions of the heavy chain (CH) of the human IgG1. Amino acids substitutions have been made in the human IgG1 Fc region in order to increase or reduce its antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

Features of pFUSE-CHIg and pFUSE2-CLlIg plasmids
- hEF1-HTLV prom is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter 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). 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.
- 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.
- βGlo pAn: The human beta-globin 3’UTR and polyadenylation sequence allows efficient arrest of the transcript transcription.

pFUSE-CHIg-hG1 specific features
- hG1e3 (Engineered human IgG1 heavy chain constant region): hG1e3 contains the following mutations E233P/L234V/L235A/ΔG236 and A327G/A330S/P331S. These mutations result in reduced binding to FcyRI and greatly reduced ADCC and CDC.
- hEF1-HTLV prom is a composite promoter comprising the elongation factor-1α (EF-1α) core promoter 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. 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.
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ANTIBODY generation using pFUSE-CHIg & pFUSE-CLIg

**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 µg/µl, 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 pFUSE-CHIg-hG1e3, the constant region of the human IgG1 heavy chain is preceded by a multiple cloning site containing four restriction sites: Eco RI, Eco RV, Xho I and Nhe I. The first three 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-hG1e3, Nhe I must be used for insertion of the 3’end of the variable region. Nhe I must be reconstituted to maintain the integrity of the constant region. Therefore we recommend to introduce by PCR an Nhe I site at the 3’ end of the variable region in frame with the constant region.

When generating the insert for VL, a Bsi WI (pFUSE2-CLIg-hk; human kappa), or AvrII (pFUSE2-CLIg-hl2; human lambda 2) 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 light 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 human lambda 2 light chain, or the human kappa light chain. pFUSE2-CLIg plasmids are selectable with blasticidin. pFUSE-CHIg plasmids are selectable with Zeocin™.

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**

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pFUSE-CH1g-hG1e3 v35
(4423 bp)

h IgG1e3 Constant Heavy
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.

<table>
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SPECIAL HANDLING

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