**pFUSEss-CHIg-hG1**

Plasmid featuring the constant region of the human IgG1 heavy chain and the IL2 signal sequence

*Catalog # pfusess-hchg1*

*For research use only*

Version # 12I25-MM

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**PRODUCT INFORMATION**

**Content:**
- 20 µg of pFUSEss-CHIg-hG1 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**
- pFUSE2ss-CL1g plasmid that features the constant region of the kappa or lambda light chains. pFUSE2ss-CL1g plasmids are selectable with blasticidin (sold separately, see RELATED PRODUCTS).
- pFUSEss-CHIg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin™.

**GENERAL PRODUCT USE**

pFUSE-CHIg and pFUSE2-CL1g 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-CHIg and pFUSE2-CL1g 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-CHIg and pFUSE2-CL1g 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 pFUSEss-CHIg and pFUSE2ss-CL1g 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 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 mRNA2.
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **CMV enh / hFerLprom:** 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.
- **IL2 ss:** The human IL2 signal sequence contains 20 amino acids (MYRMMQLSCIALRTLVTNS) and share common characteristics with signal peptides of other secretory proteins. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the immunoglobulin chain. 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 transgene transcription.

**pFUSEss-CHIg-hG1 specific features**

- **Human 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 *Streptocolloteichus hindustanus*. The same resistance gene confers selection in both mammalian cells and *E. coli*.

**References:**


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**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 µg/µl, resuspend the DNA in 20 µl of sterile H2O. Store resuspended plasmid at -20°C.

Cloning into pFUSEss-CHIg and pFUSE2ss-CLIg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSEss-CHIg-hG1, 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. Using EcoRI as the 5’ cloning site ensures that the cloned VH will follow the hIL2 signal sequence without unwanted additional amino-acids. In pFUSEss-CHIg-hG1, 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.

Note:
When generating the insert for VL, a Bsi W1 (pFUSE2ss-CLIg-hk; human kappa), or AvrII (pFUSE2ss-CLIg-hl2; human lambda 2) site must be introduced at the 3’ end. 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 pFUSEs-CLIg encoding the light chain and pFUSEss-CHIg encoding the heavy chain. Antibody production depends greatly on the ratio of heavy chain and light chain expression. Typically, pFUSEss-CHIg to pFUSE2ss-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 pFUSE2ss-CLIg and pFUSEss-CHIg respectively.

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

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

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