pFUSEss-CHIg-hD

Plasmid featuring the constant region of the human IgD (allele 2) heavy chain, and the IL2 signal sequence

Catalog code: pfusess-hchd2

For research use only

Version 24J28-MM-v37

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSEss-CHIg-hD** plasmid provided as lyophilized DNA
- 1 ml of Zeocin® (100 mg/ml)

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 Zeocin® at 4 °C or at -20 °C. The expiry date is specified on the product label.

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-CLIg plasmid that features the constant region of the kappa or lambda light chains. pFUSE2ss-CLIg 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

pFUSEss-CHIg and pFUSE2ss-CLIg 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 antibodies from Fab or scFv fragments.

pFUSEss-CHIg and pFUSE2ss-CLIg 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 pFUSEss-CHIg and pFUSE2ss-CLIg pair allows to generate an antibody that can be purified from the supernatant using the appropriate affinity chromatography.

Features of pFUSEss-CHIg and pFUSE2ss-CLIg plasmids

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] 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[2]. 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[3].
- **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.
- **IL2 ss:** The human IL2 signal sequence contains 20 amino acids (MYRMQLLSCIALSLALVTNS) 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.
- **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[4].

pFUSEss-CHIg-hD specific features

- **Human IGHD (IgD allele 2 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. 91(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

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 H_2O. 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-hD, the constant region of the human IgD heavy chain is preceded by a multiple cloning site containing five unique restriction sites: EcoRI, Eco RV, XhoI, NheI, and Eco47III. Using EcoRI as the 5' cloning site ensures that the cloned VH will follow the hIL2 signal sequence without unwanted additional amino-acids. Use Eco47III as the 3' cloning site in order to preserve the exact IgD amino acid sequence. We recommend introducing by PCR the EcoRI and Eco47III sites at the VH boundaries. Care must be taken to preserve the correct reading frame when placing the cloning sites on the variable sequence.

Note:

When generating the insert for VL, a BsiWI (pFUSE2ss-CLIg-hk; human kappa), or AvrII (pFUSE2ss-CLIg-hl2; human lambda 2) site must be introduced at the 3' end.

Antibody production

Cotransfect mammalian cells, such as 293 and CHO cells, with the recombinant plasmids pFUSE2ss-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

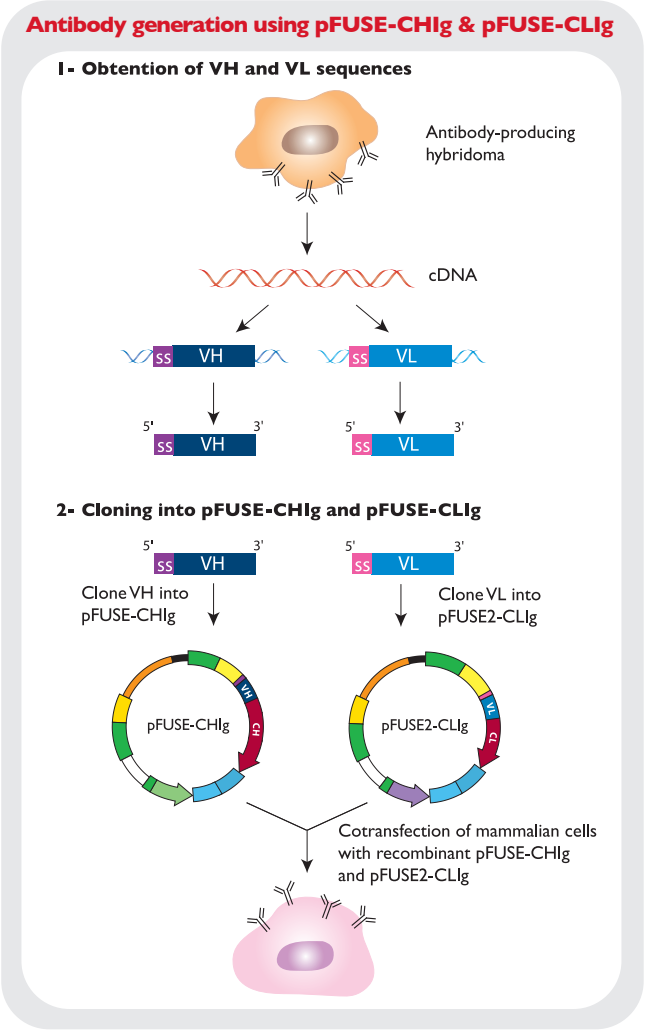
Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

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

Product	Catalog Code
pFUSE2ss-CLIg-hk	pfuse2ss-hclk
pFUSE2ss-CLIg-hl2	pfuse2ss-hcll2
LyoVec™	lyec-12
Protein L / Agarose	gel-protl-2
Zeocin®	ant-zn-1

TECHNICAL SUPPORT
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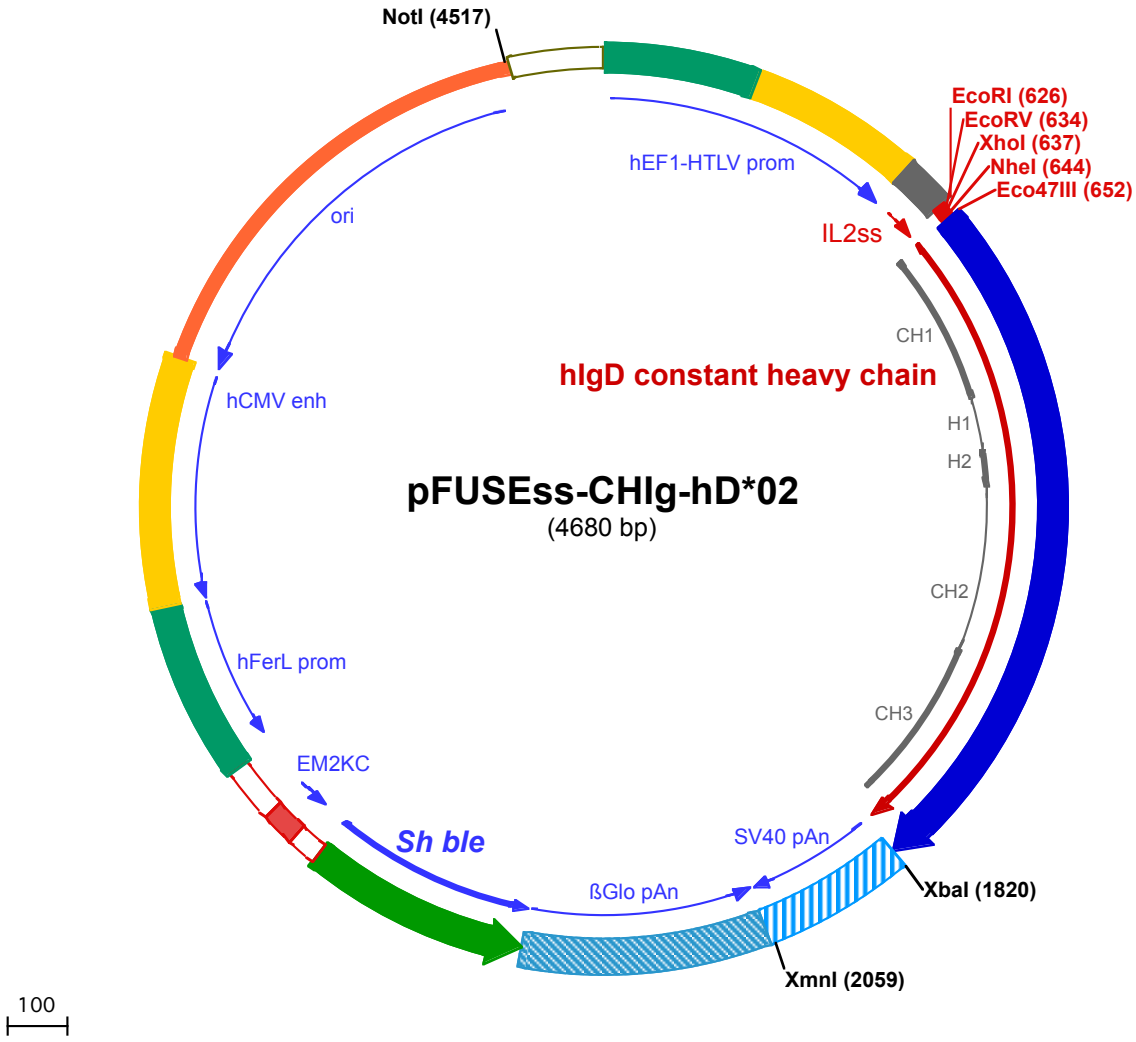
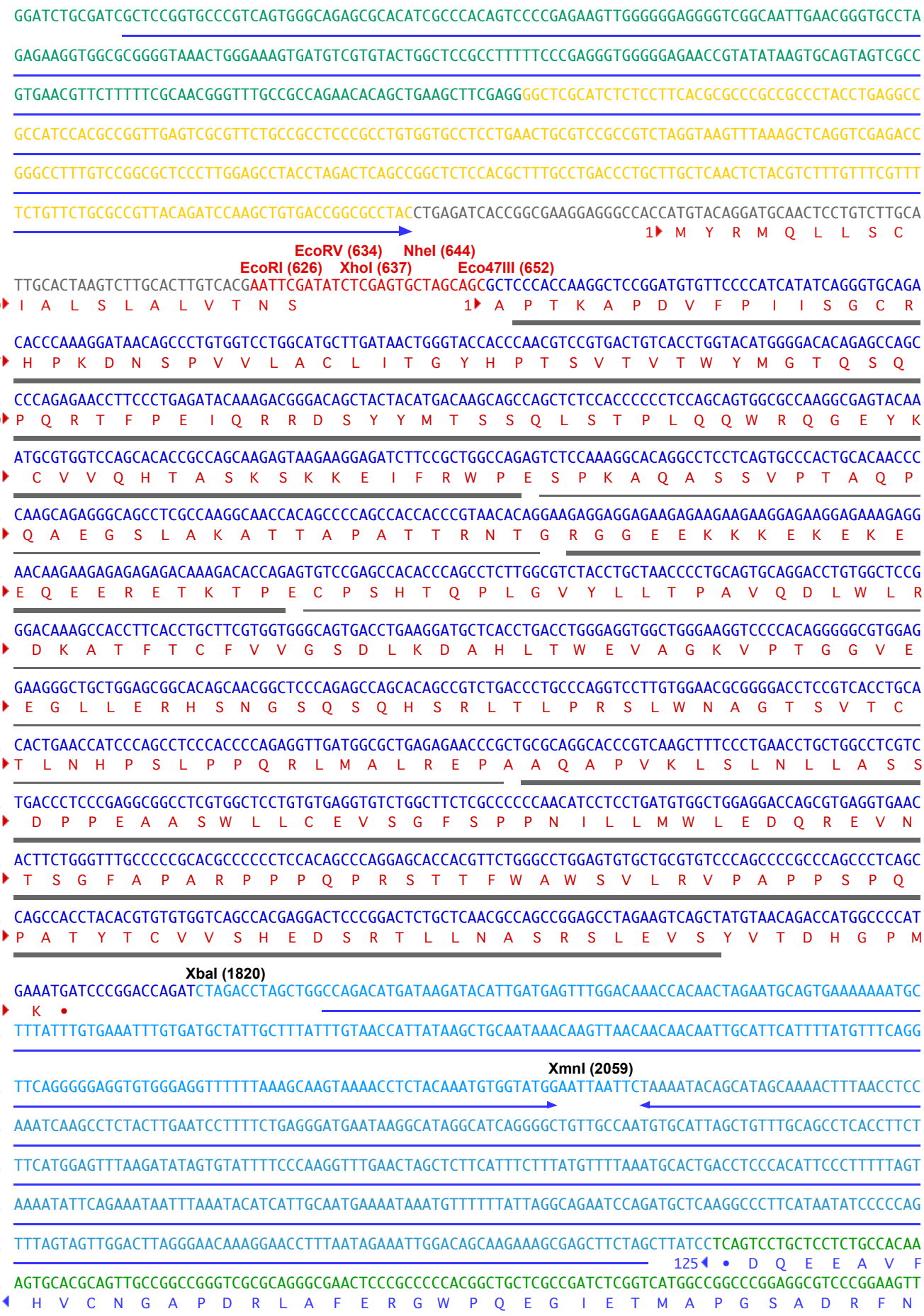

NotI (4517)
EcoRI (626)
EcoRV (634)
XhoI (637)
NheI (644)
Eco47III (652)
hEF1-HTLV prom
ori
IL2ss
CH1
hIgD constant heavy chain
hCMV enh
H1
H2
pFUSEss-CHIg-hD*02
(4680 bp)
CH2
hFerL prom
CH3
EM2KC
Sh ble
SV40 pAn
ßGlo pAn
XbaI (1820)
XmnI (2059)
100


EcoRV (634)
NheI (644)
EcoRI (626)
XhoI (637)
Eco47III (652)
XbaI (1820)
XmnI (2059)

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  84  T  S  V  V  E  S  W  E  A  Y  L  E  D  L  G  R  V  W  V  W  A  L  T  N  D  P  V  V  Q  D  Q  V  A
2701 CTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTC
  50  S  I  F  L  T  V  D  D  R  V  V  G  A  F  D  D  E  V  F  D  R  S  F  G  L  R  D  T  W  F  E  V  A  G
2801 CGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCctgtcaggagaggaaagagaagaaggttagtacaatt
  17  A  V  D  R  A  T  L  V  P  V  A  S  T  L  K  A  M
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                    NotI (4517)
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