pBOOST4-mFLT3L-mGMCSF

Genetic adjuvant for DNA vaccines featuring the murine mFLT3L and GM-CSF genes

Catalog code: pbst4-mf3csf2

https://www.invivogen.com/pboost4-mflt3l-mgmcsf

For research use only

Version 21F28-MM

PRODUCT INFORMATION

Contents

- 20 µg of pBOOST4-mFLT3L-mGMCSF provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store blasticidin at 4°C or -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 and lyophilized.

GENERAL PRODUCT USE

pBOOST4 plasmids were developed as genetic adjuvants for DNA vaccines to potentiate the immune response to a specific antigen. They contain two transcription units allowing the co-expression of two cytokine genes that promote dendritic cell development. These plasmids feature two strong composite promoters derived from the ferritin light chain (FerL) and heavy chain (FerH) core promoters. Both promoters work concomitantly to express ferritin, a ubiquitous protein, therefore, eliminating potential transcription interference. The genes cloned into pBOOST4 comprise the coding sequence (without introns) from the ATG to the Stop codon.

PLASMID FEATURES

- **Murine** FLT3L (699 bp) / **Murine** GM-CSF (426 bp)

Fms-like tyrosine kinase 3 ligand (FLT3L) is a synergistic hematopoietic growth factor that mediates cell survival, cell proliferation, and differentiation of dendritic cells[1]. Granulocyte-macrophage colony-stimulating factor (GM-CSF), also known as colony-stimulating factor 2 (CSF2), is an important hematopoietic growth factor and immune modulator[2]. GM-CSF is widely used in genetic immunization studies to increase antibody titer. Notably, it has been reported that mice co-immunized with both the FLT3L and GM-CSF plasmids had approximately a nine-fold higher antibody titer[3].

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein[4]. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.
- **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[5].
- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (hCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The hCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer[6].
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell *et al.*[7]
- **ori pMB1:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression[8].
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES.
- **hEF1a pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

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.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

Intramuscular inoculation

Plasmid DNA solution

1. Prepare the vaccine plasmid solution by resuspending 10 µg of the vaccine plasmid DNA in 50 µl saline solution.
2. Prepare the pBOOST4 solution by mixing 10 µg of pBOOST4-mFLT3L-mGMCSF and 90 µg of the mock plasmid pBOOST4-mcs in 50 µl saline solution for low dose, or 100 µg of pBOOST4-mFLT3L-mGMCSF in 50 µl saline solution for high dose.
3. Combine both solutions to obtain a total of 110 µg DNA in 100 µl saline solution.

Note: The quantities are per mouse.

Intramuscular injections

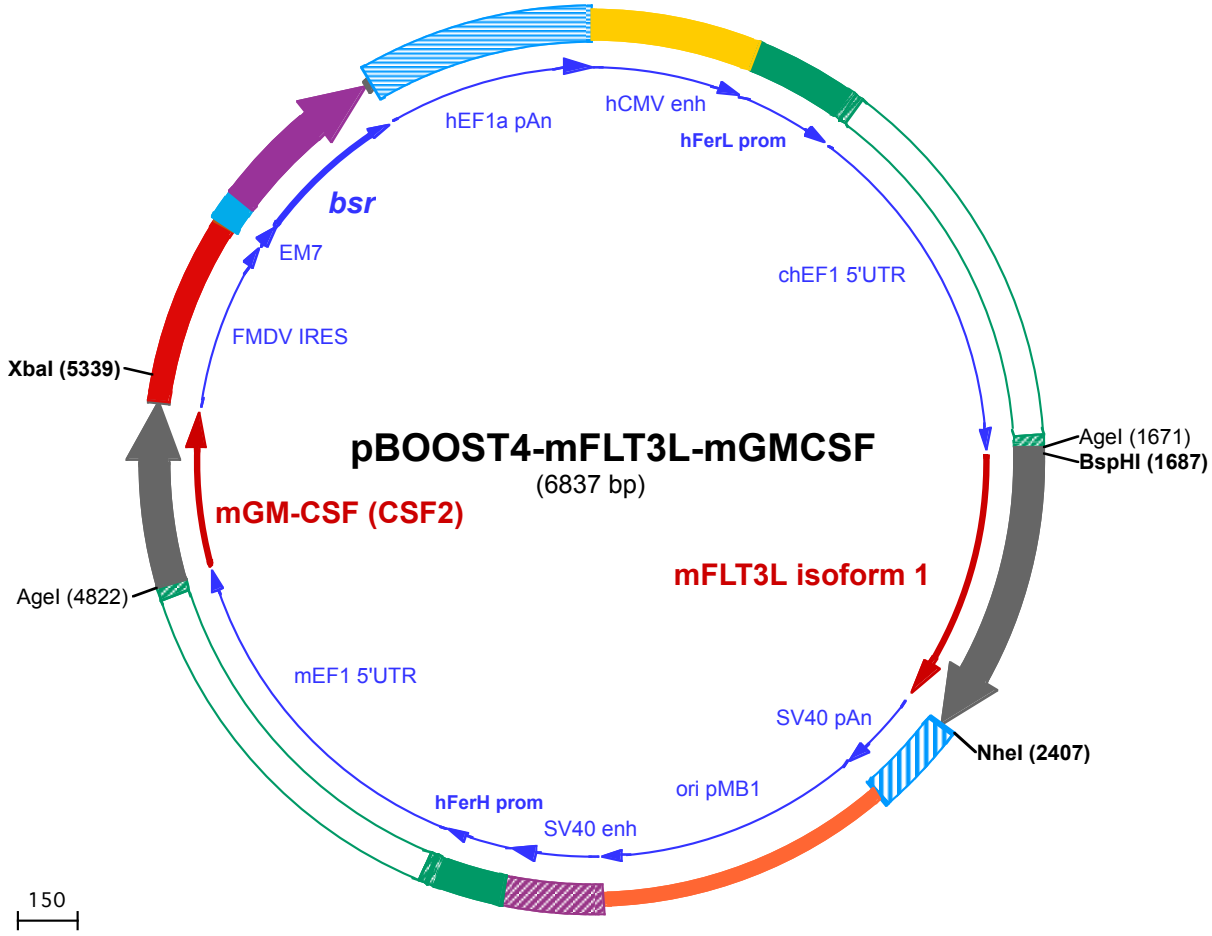
1. Inoculate 6 to 8-week old female BALB/c mice with 100 µl plasmid DNA solution (described above) into the quadriceps at 0 and 4 weeks.
2. Collect sera and analyze for antibodies at 8 weeks.

RELATED PRODUCTS

Product	Description	Cat.Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11
pBOOST4-mcs	Negative Control	pbst4-mcs

REFERENCES

1. Shao Z. *et al.*, 2009. Fms-like tyrosine kinase 3 ligand regulates migratory pattern and antigen uptake of lung dendritic cell subsets in a murine model of allergic airway inflammation. J Immunol. 183(11):7531-8. **2. Shi Y. *et al.*, 2006.** Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. Cell Res. 16(2):126-33. **3. Chambers R.S. & Johnston S.A., 2003.** High-level generation of polyclonal antibodies by genetic immunization. Nat Biotechnol. 21(9):1088-92. **4. Eisenstein RS. & Munro H.N. 1990.** Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58. **5. Dean D.A. *et al.*, 1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **6. Boshart M. *et al.*, 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 141(2):521-30. **7. Carswell S. & Alwine J.C. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10: 4248-4258. **8. Ramesh N. *et al.*, 1996.** High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24(14):2697-700.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hCMV enh
hFerL prom
bsr
EM7
chEF1 5'UTR
FMDV IRES
XbaI (5339)
AgeI (1671)
BspHI (1687)
mGM-CSF (CSF2)
mFLT3L isoform 1
AgeI (4822)
mEF1 5'UTR
SV40 pAn
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SV40 enh
150


AgeI (1671)
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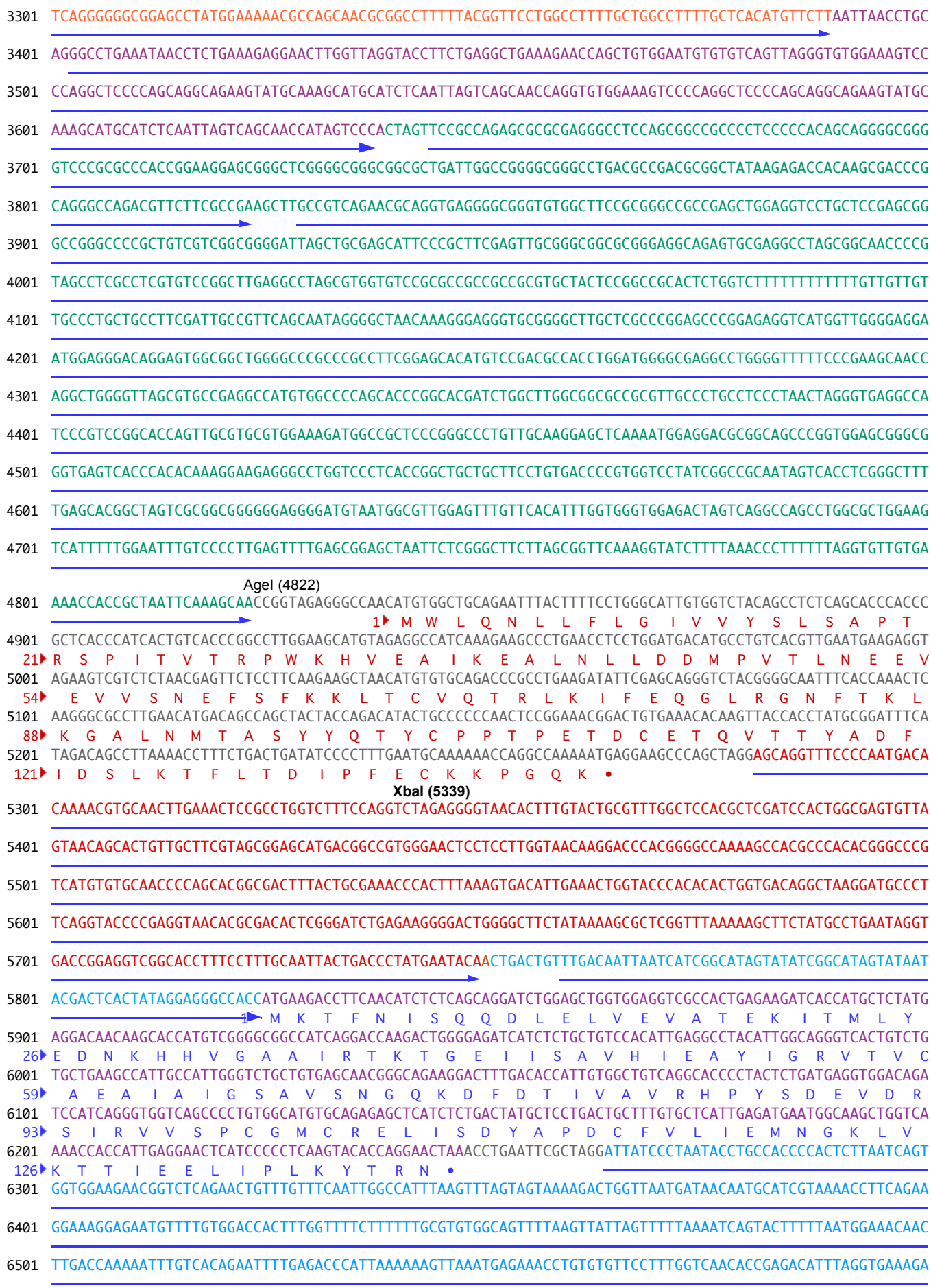
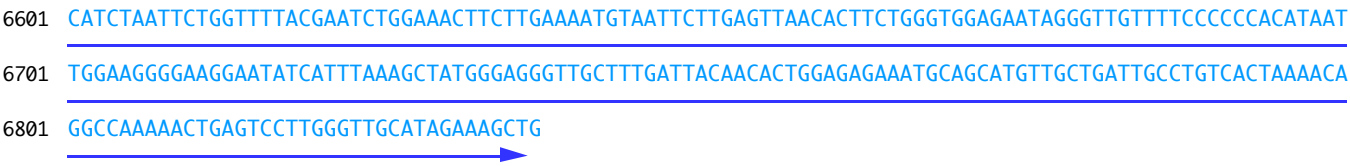
NheI (2407)


AgeI (4822)
XbaI (5339)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