pFUSE-rtIgG2B-Fc1

Plasmid designed for the construction of rat IgG2B Fc-Fusion proteins
Catalog # pfuse-rtg2bfc1

For research use only
Version # 06120-SV

PRODUCT INFORMATION

Content:
- 20 µg of pFUSE-rtIgG2B-Fc1 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 and lyophilized.

GENERAL PRODUCT USE

pFUSE-Fc is a family of plasmid developed to facilitate the construction of Fc-Fusion proteins by fusing a sequence encoding a given protein to the Fc region of an immunoglobulin.

pFUSE-Fc plasmids yield high levels of Fc-Fusion proteins. The level of expression is usually in the µg/mL range. They can be transfected in a variety of mammalian cells, including myeloma cell lines, CHO cells, monkey COS cells and human embryonic kidney (HEK)293 cells. These cells are commonly used in protein purification systems.

pFUSE-Fc2 (IL2ss) plasmids allow the secretion of Fc-Fusion proteins. They contain the IL2 signal sequence (IL2ss) for the generation of Fc-Fusion proteins derived from proteins that are not naturally secreted. As Fc-Fusion proteins are secreted, they can be easily detected in the supernatant of pFUSE-Fc-transfected cells by SDS-PAGE. Furthermore, functional domains can be identified by immunoblotting and ligand blotting. Fc-Fusion proteins can be easily purified by single-step protein A or protein G affinity chromatography.

PLASMID FEATURES

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter and the R segment and part of the US 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.

- **rtIgG2B Fc (rat)**: The Fc region comprises the CH2 and CH3 domains of the IgG2B heavy chain and the hinge region. The hinge serves as a flexible spacer between the two parts of the Fc-Fusion protein, allowing each part of the molecule to function independently.

- **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.

- **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.

- **βGlo pAn**: The human beta-globin 3’UTR and polyadenylation sequence allows efficient arrest of the transgene transcription.

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 H2O. Store resuspended plasmid at -20°C.

**Selection of bacteria with E. coli Fast-Media®**
Fast-Media® is a fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic. Fast-Media® Zeo is available separately: #fas-zn-l (liquid), #fas-zn-s (agar).

**Method:**
1- Pour the contents of a Fast-Media® pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask
3- Heat in a microwave on MEDIUM power setting (about 400 Watts), until bubbles start appearing (approximately 3 minutes).
4- Swirl gently to mix the preparation.
5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

**Note:** Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

References:
pFUSE-rtIgG2B-Fc1
(4130 bp)