**PRODUCT INFORMATION**

**Content:**
- 20 µg of pFUSE-mlIgG1e2-Fc2 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 the effector region of a protein to the Fc region of an immunoglobulin G (IgG). 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, cells that 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.

InvivoGen provides pFUSE-Fc vectors featuring Fc regions from different species and isotypes. Four murine isotypes are available: IgG1, IgG2a, IgG2b and IgG3. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity (CDC). In ADCC, the Fc region of an antibody binds to Fc receptors (FcγRs) on the surface of immune effector cells such as natural killers and macrophages, leading to the phagocytosis or lysis of the targeted cells. In CDC, the antibodies kill the target cells by triggering the complement cascade at the cell surface of G isoforms exert different levels of effector functions increasing in the order of mlG1<mlG3<mlG2a.

Under certain circumstances, for example when depletion of the target cell is undesirable, abrogating effector functions is required. On the contrary, in the case of antibodies intended for oncology use, increasing effector functions may improve their therapeutic activity. Modifying effector functions can be achieved by engineering the Fc regions to either improve or reduce their binding to FcγRs or the complement factors.

**PLASMID FEATURES**

- **mlG1e2 Fc (mouse IgG1 engineered Fc):** The Fc region comprises the CH2 and CH3 domains of the IgG 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. The Fc region of mouse IgG1 mediates low CDC and no ADCC. This engineered form of mlG1 contains the T252M mutation that increases affinity to protein A, thus facilitating affinity column purification.
- **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.
- **IL2 ss:** The IL2 signal sequence contains 21 amino acids and share common characteristics with signal peptides of other secretory proteins. The intracelluar cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the antigenic protein.
- **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.
- **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.
- **fGlo 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).

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 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
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.

**RELATED PRODUCTS**

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<thead>
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</tr>
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<td>ant-zn-l</td>
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<tr>
<td>Fast-Media® Zeo TB</td>
<td>fas-zn-l</td>
</tr>
<tr>
<td>Fast-Media® Zeo Agar</td>
<td>fas-zn-s</td>
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mIgG1-Fc [T252M]

pFUSE-mIgG1e2-Fc2
(4193 bp)