**pFUSE-mlIgG2Ae1-Fc2**

Plasmid containing a mouse engineered IgG2A Fc region

**Catalog #: pfc2-mg2ae1**

**For research use only**

**Version 20K05-MM**

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**PRODUCT INFORMATION**

**Content:**
- 20 µg of pFUSE-mlIgG2Ae1-Fc2 (IL2ss) 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 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. Three murine isotypes are available: IgG1, IgG2a and IgG3. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). IgG isoforms exert different levels of effector functions increasing in the order of mIgG1<mIgG3<mIgG2a. 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 is required. The engineered form mIgG2ae1 Fc was engineered by mutating the amino acids that are critical for FcγRs and C1q binding. The engineered form mIgG2ae1 contains the following mutations: L235E and E318A/K320A/K322A.

**PLASMID FEATURES**

- **mlG2Ae1 Fc (mouse IgG2A 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 IgG2a mediates high ADCC and CDC. To reduce its cytotoxicity, mIgG2a Fc was engineered by mutating the amino acids that are critical for FcγRs and C1q binding. The engineered form mIgG2ae1 contains the following mutations: L235E and E318A/K320A/K322A.
- **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 20 amino acids 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 antigenic protein.
- **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 Streptototaleichus 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.

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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 H₂O. Store resuspended plasmid at -20 °C.

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

**Zeocin™ usage**
This antibiotic can be used for *E. coli* at 25 μg/ml in liquid or solid media and at 50-200 μg/ml to select Zeocin™-resistant mammalian cells.

**RELATED PRODUCTS**

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<th>Product</th>
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<tr>
<td>Zeocin™</td>
<td>ant-zn-1</td>
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pFUSE-mIgG2Ae1-Fc2

(4212 bp)