pFUSE-hIgG1e3-Fc2
Plasmid containing a human engineered IgG1 Fc region
Catalog # pfc2-hg1e3
For research use only
Version # 11E10-3C

PRODUCT INFORMATION
Content:
- 20 µg of pFUSE-hlgG1e3-Fc2 (IL2ss) 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 up to 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, 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. In humans, there are four isotypes: IgG1, IgG2, IgG3 and IgG4. The Fc region mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). IgG isozymes exert different levels of effector functions increasing in the order of IgG4<lgG2<lgG1<lgG3. Human IgG1 dislays high ADCC and CDC, and is the most suitable for therapeutic use against pathogens and cancer cells.

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. Amino acids substitutions have been made in the human IgG1 Fc region in order to increase or reduce its ADCC and CDC.

PLASMID FEATURES
• hlgG1e3-Fc (human 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.

IgG1 Fc binds with a high affinity to the activating Fc receptor FcγRI while IgG2 and IgG4 bind with low affinity. Substitution into human IgG1 of IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 greatly reduced ADCC and CDC. IgG1 contains the hinge and residues at positions 233-236 from IgG2. Substitutions at positions 327, 330 and 331 were performed by PCR.

• hEF1-HTLV prom is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter1 and the R segment and part of the U5 sequence (R-U5′) of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat2. 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 7 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.

• 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 Streptomyces lividans. The same resistance gene confers selection in both mammalian cells and E. coli.

• bGlo pAn: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription.

3. Shields RL. et al., 2001. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem. 276(9):6591-604.
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$_2$O. 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

<table>
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<tr>
<th>Product</th>
<th>Catalog Code</th>
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<tr>
<td>Zeocin™</td>
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<tr>
<td>Fast-Media® Zeo TB</td>
<td>fas-zn-l</td>
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<tr>
<td>Fast-Media® Zeo Agar</td>
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**Fast-Media®**

Microwaveable media for selection and propagation of *E. coli* transformants

Catalog # fas-xx-l, fas-xx-s, fas-xx-xgal

For research use only

Version # 10G07-MM

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**PRODUCT INFORMATION**

**Contents:**

*E. coli* Fast-Media® are prepared as individual sealed pouches containing the necessary amount of powder for preparation of 200 ml of selective liquid or agar medium.

30 pouches are supplied for each order of TB or Agar and 20 pouches are supplied for each order of XGal Agar.

**Storage and stability:**

Fast-Media® are shipped at room temperature, and must be stored in a dry and cool place. They are stable for 2 years at room temperature.

When properly prepared, Fast-Media® plates or broths are stable for 4 weeks at 4°C, and remain sterile and selective.

**Quality control:**

The high quality and performance of each formulation has been tested with some widely used and proprietary *E. coli* K12 derived strains*. These include DH5α, Top10, MC1061, XL1 blue, JM 109, TB1, GT100, GT110, GT115, GT116.

The adequate plasmids carrying the appropriate *E. coli* resistance genes are used as positive control.

*E. coli* recipient strains carrying the Tn5 transposon are resistant to Kanamycin and Zeocin™.

**GENERAL PRODUCT USE**

*E. coli* Fast-Media® are microwaveable ready-to-use solid or liquid media, supplied with a selective antibiotic, and chromogenic substrates (for five references), therefore designed for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies.

- **Fast-Media® Agar** formulation is LB based agar medium supplemented with selective antibiotic, it is used for selection of resistant *E. coli* colonies after transformation by vectors carrying a selection resistance gene.

- **Fast-Media® X-Gal** formulation is a LB based agar medium supplemented with selective antibiotic, X-Gal and IPTG. It is used for detection of blue/white resistant colonies after transformation by a vector carrying LacZ gene.

- **Fast-Media® TB** formulation is a Terrific Broth based liquid medium supplemented with selective antibiotic. It’s used for high cell density culture of transformed bacteria, and extraction of high quantity and quality of required plasmid.

**FAST-MEDIA® FEATURES**

*E. coli* Fast-Media® offer researchers a quick and convenient way to prepare 200 ml of liquid culture medium, or 8-10 agar plates in about five minutes USING A MICROWAVE INSTEAD OF AN AUTOCLAVE.

**E. coli** Fast-Media® are available with a large variety of prokaryotic selective agents including Ampicillin, Blasticidin S, Hygromycin B, Kanamycin, Puromycin and Zeocin™ (see table below). Fast-Media® is also available with no selective agent (Base) that can be prepared with or without antibiotics.

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**SPECIAL HANDLING**

Caution should be exercised during handling of Fast-Media® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breach the dust.

**METHOD**

For customer convenience, procedure is directly printed on each pouch.

1- Pour the pouch contents into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled or deionized water.
3- Mix thoroughly by swirling the glass bottle or flask.
4- Heat in a microwave oven on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes).
5- Do not heat in a closed container.
6- Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.
7- Allow the medium to cool to 50-55 °C, use directly for liquid medium, or pour plates for solid medium.

**Caution:** Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.

**Note:** Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.

**For preparation of supplemented Fast-Media® Base.**

- Follow the instructions above and when media has cooled to 50-55 °C add the antibiotic at the appropriate concentration for selection of *E. coli*.

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**TECHNICAL SUPPORT**

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