pFUSE-mIgG2Ae1-Fc1

Plasmid containing a mouse engineered IgG2a Fc region

Catalog # pfc1-mg2ae1

For research use only

Version # 08H26-SV

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE-mIgG2Ae1-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 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-Fc plasmids allow the secretion of Fc-Fusion proteins. 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 may improve their therapeutic activity[1]. 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 mouse IgG2a Fc region in order to reduce its ADCC and CDC.

PLASMID FEATURES

• mIgG2Ae1 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[2].

• hEF1-HTLV prom is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[3] 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[4]. 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.

• SV40 pAn: the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[5].

• 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*.

• ßGlo pAn: The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[6].

1. Carter PJ., 2006. Potent antibody therapeutics by design. Nature Reviews Immunology. Advance online publication.
2. Steurer W. *et al.*, 1995. Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. J Immunol. 155(3):1165-74.
3. Kim DW *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23.
4. Takebe Y. *et al.* 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72.
5. Carswell S. & Alwine JC. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58.
6. Yu J. & Russell JE. 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

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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.

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

Product	Catalog Code
Zeocin™	ant-zn-1
Fast-Media® Zeo TB	fas-zn-l
Fast-Media® Zeo Agar	fas-zn-s

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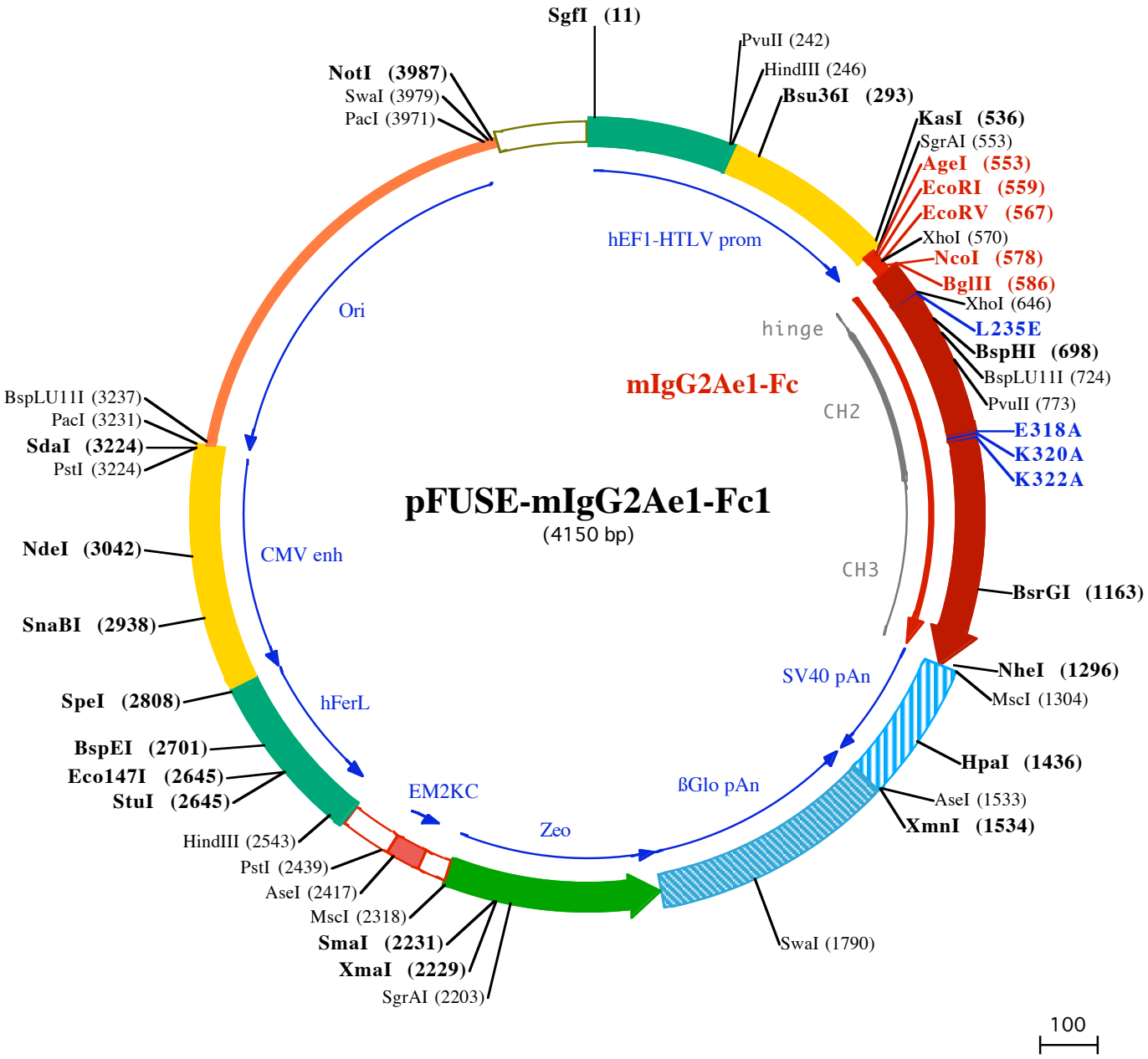
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mIgG2Ae1-Fc
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CH3
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CMV enh
hFerL
EM2KC
Zeo
ßGlo pAn
SV40 pAn
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PvuII (242)
HindIII (246)
Bsu36I (293)
KasI (536)
SgrAI (553)
AgeI (553)
EcoRI (559)
EcoRV (567)
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NcoI (578)
BglII (586)
XhoI (646)
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BspHI (698)
BspLU11I (724)
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K320A
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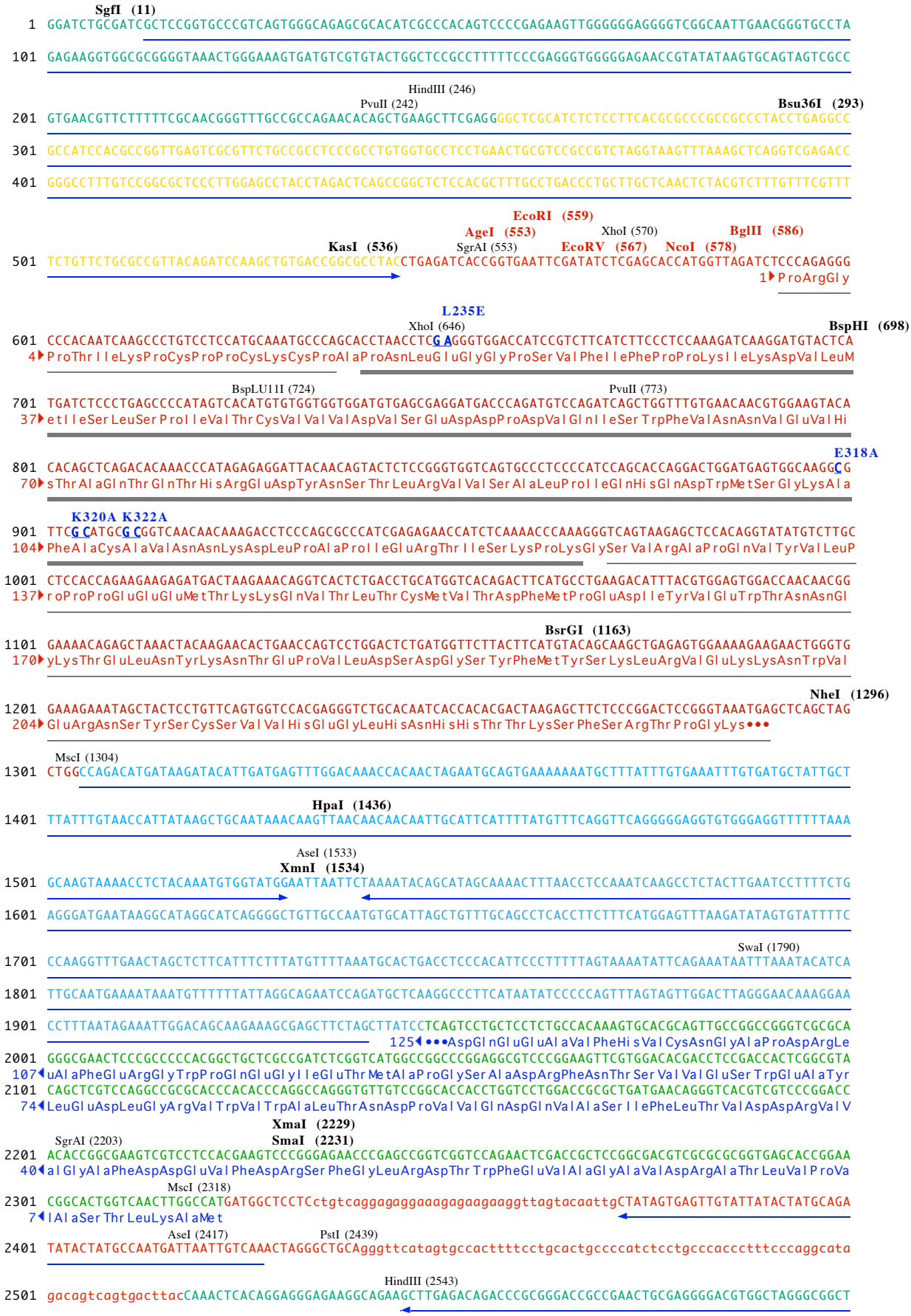
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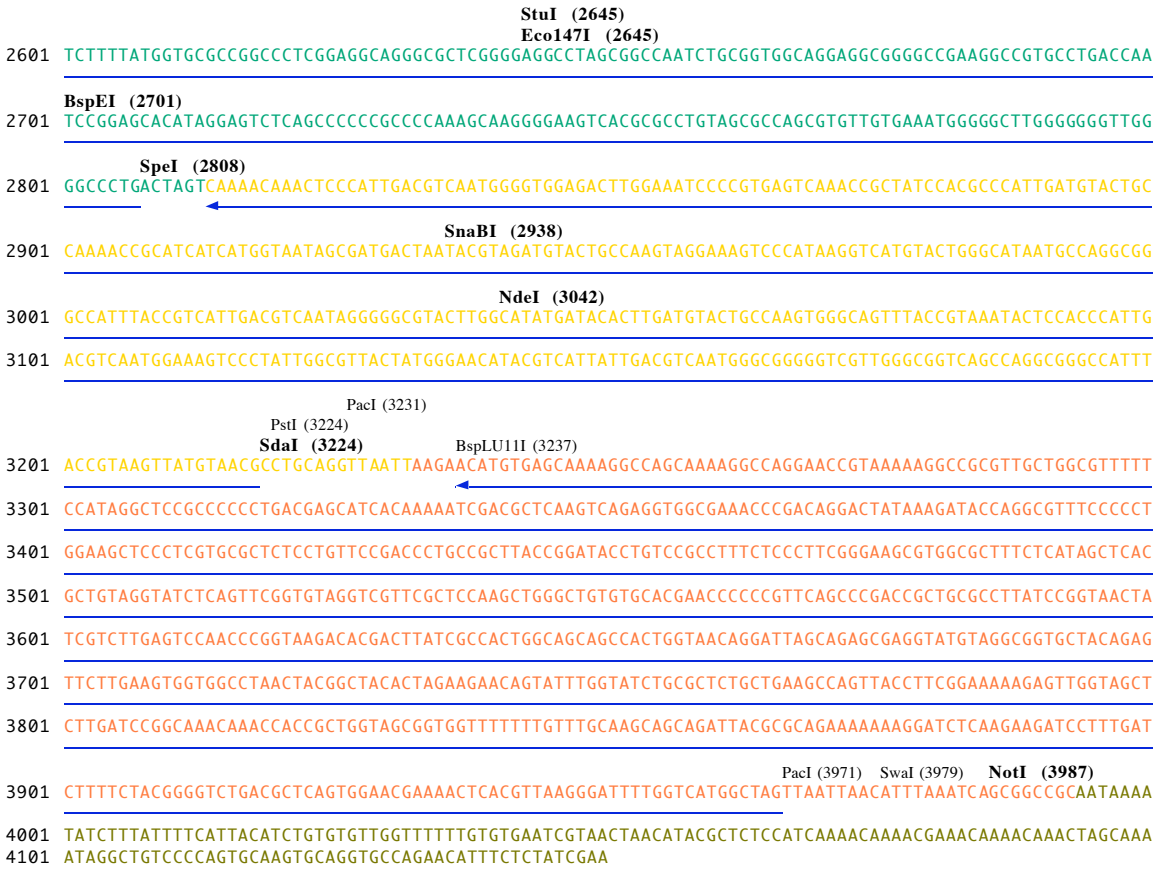
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SnaBI (2938)
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