pUNO1Fc-SARS2-N

Plasmid designed for the production of the SARS-CoV-2 Nucleocapsid::Fc fusion protein

Catalog code: p1fc-cov2-n

https://invivogen.com/sars2-nucleocapsid-tag-production-vectors

For research use only

Version 20H28-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized plasmid DNA
- 2 x 1 ml Blasticidin at 10 mg/ml

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year.
- Store Blasticidin at 4°C or -20°C. The expiry date is specified on the product label.

Quality control

- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.
- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.

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 water.
- Store resuspended plasmid at -20°C.

- **Plasmid amplification and cloning**

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

- **Blasticidin usage**

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells.

PLASMID FEATURES

SARS-CoV-2 Nucleocapsid::hIgG1-Fc cassette

- **SV40 enhancer** is comprised of a 72-base-pair repeat and allows the enhancement of gene expression in a wide range of hosts. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[1].
- **EF-1α/HTLV composite promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[2] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV)[3]. EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.
- SARS-CoV-2 Nucleocapsid ORF

Nucleocapsid (N) is a phosphoprotein that associates with the viral RNA genome and forms the ribonucleoprotein core. N has a number of distinct domains, including an N-terminal RNA-binding domain, which captures the RNA, a C-terminal dimerization domain, which acts as an anchor to the replication-transcription complexes (RTCs) and, an intrinsically disordered central Ser/Arg (SR)-rich linker for primary phosphorylation[4,5]. The N protein is highly immunogenic and abundantly expressed during infection, and is thus, capable of inducing protective immune responses against SARS-CoV-2. The pUNO1Fc-SARS2-N plasmid contains the nucleocapsid coding sequence from the Wuhan-Hu-1 isolate, with optimized signal sequence and codon usage.

- **TEV sequence** (Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser)) is cleavable by the TEV (Tobbaco Etch Virus) protease between Gln and Gly/Ser residues.
- **Fc** is the Fc region of human IgG1 cloned at the C-terminus of the nucleocapsid and is followed by a stop codon. This tag 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.
- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA[6].

Antibiotic Selection cassette

- **hCMV (human cytomegalovirus) enhancer & promoter** drives the expression of the blasticidin resistance in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- ***bsr* (blasticidin resistance gene)** from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and *E. coli* transformants.
- **Human β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference[7] and possible recombination events with the SV40 pAn signal.

General features of pUNO1Fc-SARS2-N

- **pMB1 ori** is a minimal *E. coli* origin of replication.

REFERENCES

1. Dean DA. *et al.* 1999. Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **2. Kim D. *et al.*, 1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 91(2):217-23. **3. 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. **4. Kang, S. *et al.* 2020.** Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. Acta Pharm Sin B. **5. Khan, M.T. *et al.* 2020.** SARS-CoV-2 nucleocapsid and Nsp3 binding: an in silico study. Arch Microbiol. **6. Carswell S. & Alwine J., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. **7. Yu J. & Russell J., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. Mol Cell Biol. 21(17):5879-88.

TECHNICAL SUPPORT

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GENERAL PRODUCT USE

Please note: Due to the lack of a unique restriction site at the 3' end of the fusion gene, this plasmid does not facilitate the subcloning of the Fc-tagged nucleocapsid gene.

• **Stable gene expression in mammalian cells.** pUNO1 plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pUNO1 plasmids contain the blasticidin-resistance gene (*bsr*) driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in *E. coli*, as well as the selection of stable clones in mammalian cells using the same selective antibiotic. pUNO1 plasmids facilitate high levels of expression and secretion of the gene product.

• **Detection and purification of the nucleocapsid protein.** The pUNO1Fc-SARS2-N plasmid has been designed to generate the nucleocapsid with a C-terminal human IgG1-Fc (Fc) tag to facilitate the detection of the secreted protein with an anti-Fc antibody, and its purification using protein G affinity resins.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11
Protein G / Agarose	Ig binding protein	gel-agg-2
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
pUNO1-hTMPRSS2b	Expression vector	puno1-htp2b
pUNO1His-SARS2-S	Production vector	p1his-cov2-s
pUNO1Fc-SARS2-S	Production vector	p1fc-cov2-s
pUNO1His-SARS2-S1	Production vector	p1his-cov2-s1
pUNO1His-SARS2-RBD	Production vector	p1his-cov2-rbd
pUNO1Fc-SARS2-RBD	Production vector	p1fc-cov2-rbd
pUNO1His-SARS2-N	Production vector	p1his-cov2-n