pUNO1His-SARS2-RBD

Plasmid designed for the production of the SARS-CoV-2 Spike RBD::His fusion protein

Catalog code: p1his-cov2-rbd

https://invivogen.com/sars2-spike-rbd-tag-production-vectors

For research use only

Version 20E26-NJ

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 at least for 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.

GENERAL PRODUCT USE

- **Subclone gene into another vector.** Two unique restriction sites flank the gene, allowing convenient excision. The 5' site is AgeI which is compatible with XmaI, BspEI, NgoMIV, and SgrAI. The 3' site is NheI which is compatible with XbaI, SpeI, and AvrII.

- **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 allows high levels of expression and secretion of the gene product.

- **Detection and purification of the encoded protein.** pUNO1His-SARS2-RBD plasmid has been designed to generate the Spike protein RBD in mammalian cells with the polyhistidine (His) tag in C-terminus in order to facilitate the detection of the secreted protein with an anti-His antibody, and its purification using an NI-NTA column.

PLASMID FEATURES

- **SARS-CoV-2 Spike RBD::His**

ORF size: 756 bp

Spikes are multifunctional glycoproteins that mediate the entry of coronaviruses into the target cell and are critical determinants of the viral host and tissue tropism. Spikes exhibit a large ectodomain comprised of two subunits. The S1 subunit contains the ACE2 receptor binding domain (RBD), while the S2 subunit features the elements mediating the fusion of viral and host membranes[1-4]. Protein vaccination studies using the full Spike or its S1 or RBD fragments have provided encouraging results to protect from SARS-CoV and MERS-CoV[5,6]. The pUNO1His-SARS2-RBD plasmid contains the Spike RBD coding sequence of the SARS-CoV-2 Wuhan-Hu-1 (D614) isolate, with optimized signal sequence and codon usage.

- **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[7].
- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[8] and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). 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[9] 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.
- **His** is a polyhistidine tag cloned at the C-terminus of the gene of interest and followed by a Stop codon.
- **SV40 pAn** is he Simian Virus 40 late polyadenylation (pAn) signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA[10].
- **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **hCMV (human cytomegalovirus) enhancer & promoter** drive 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*.

TECHNICAL SUPPORT
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• ***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 beta-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference[11] and possible recombination events with the SV40 pAn signal.

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. Blasticidin is supplied as a 10 mg/ml colorless solution in HEPES buffer.

REFERENCES

1. Li F., 2016. Structure, function, and evolution of coronavirus spike proteins. Annu. Rev. Virol. 3:237-261. **2. Li F. *et al.*, 2005.** Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 309:1864-1868. **3. Walls A.C. *et al.*, 2020.** Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 181(2):281-292.e6. **4. Hoffmann M. *et al.*, 2020.** SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 181:1-16. **5. Wang N. *et al.*, 2020.** Subunit vaccines against emerging pathogenic human coronaviruses. Front. Microbiol. 11:298. DOI: 10.3389/fmicb.2020.00298. **6. Padron-Regalado E., 2020.** Vaccines for SARS-CoV-2: Lessons from other coronavirus strains. Infect. Dis. Ther. DOI: 10.1007/s40121-020-00300-x. **7. Dean DA. *et al.* 1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **8. 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. **9. 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. **10. 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. **11. 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.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent *E. coli*	gt116-11
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
pUNO1-hTMPRSS2b	Expression vector	puno1-htp2b
pUNO1Fc-SARS2-S	Production vector	p1fc-cov2-s
pUNO1His-SARS2-S	Production vector	p1his-cov2-s
pUNO1Fc-SARS2-S1	Production vector	p1fc-cov2-s1
pUNO1His-SARS2-S1	Production vector	p1his-cov2-s1
pUNO1Fc-SARS2-RBD	Production vector	p1fc-cov2-rbd

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