pLV-SARS2-S-d19

Expression vector for lentiviral pseudotyping with a truncated (Δ19) SARS-CoV-2 Spike (D614)

Catalog code: plv-cov2-sd19

https://www.invivogen.com/sars2-spike-lentiviral-expression-vectors

For research use only

Version 20K02-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized plasmid DNA

Storage and Stability

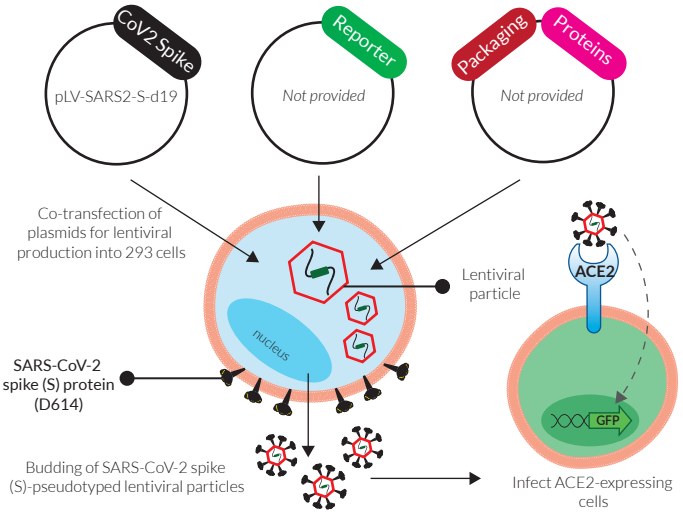
- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year.

Quality control

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

APPLICATION

pLV-SARS2-S-d19 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 spike protein. The basic strategy involves transfecting 293T cells with a lentiviral backbone plasmid encoding a fluorescent or luminescent reporter protein (e.g. GFP), a plasmid expressing the minimal set of lentiviral proteins necessary to assemble viral particles, and InvivoGen's pLV-SARS2-S-d19. The transfected cells produce SARS-CoV-2 Spike-pseudotyped lentiviral particles, which can then be used to infect permissive cells that express the host receptor, ACE2.



SARS-CoV-2 spike (S)-pseudotyped lentiviral production

PLASMID FEATURES

- SARS-CoV-2 D614-Spike (Δ19 truncated) ORF
  - Original spike **D614 isolate**
  - **Truncated SARS-CoV-2 spike ORF** with the last 19 amino acids (d19), which contain the ER-retention motif, removed. This has been shown to improve the expression of the S protein, particularly in pseudovirions[1,2].

Spike (S) is a structural glycoprotein expressed on the surface of SARS-CoV-2. It mediates membrane fusion and viral entry into target cells upon binding to the host receptor ACE2 and its cleavage by the cellular protease, TMPRSS2[3]. The S protein consists of an N-terminal ectodomain, a transmembrane anchor, and a short C-terminal cytoplasmic tail. The ectodomain contains the S1 subunit, which encodes the receptor binding domain (RBD), a key target in treatment and vaccination strategies against COVID-19, as well as the S2 subunit, needed for membrane fusion[4]. Notably, the C-terminal cytoplasmic tail of the S protein encodes a presumptive endoplasmic reticulum (ER)-retention motif (KxHxx), which has previously been shown to enable the accumulation of SARS-CoV S proteins at the ER-Golgi intermediate compartment (ERGIC) and facilitate their incorporation into new virions[5].

- **hCMV (human cytomegalovirus) enhancer & promoter** drives high expression of the SARS-CoV-2 spike gene in mammalian cells.
- **Rabbit (rbt) β-Globin intron** enhances the expression of the SARS-CoV-2 spike gene in mammalian cells.
- **Rabbit β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of the SARS-CoV-2 spike gene. It allows efficient transcription termination and polyadenylation of the mRNA.
- ***bla* (Ampicillin resistance gene)** encodes the β-lactamase enzyme, which confers resistance to the antibiotic ampicillin. Therefore, ampicillin can be used to select stable clones in mammalian cells and *E. coli* transformants.
- **pMB1 ori** is a minimal *E. coli* origin of replication.

REFERENCES

1. Johnson, M.C. *et al.* 2020. Optimized pseudotyping conditions for the SARS-COV2 Spike glycoprotein. bioRxiv. **2. Ou, X. *et al.* 2020.** Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11, 1620. **3. 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. **4. Walls A.C., *et al.*, 2020.** Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 181(2):281-292.e6. **5. Ujike, M. *et al.* 2016.** The contribution of the cytoplasmic retrieval signal of severe acute respiratory syndrome coronavirus to intracellular accumulation of S proteins and incorporation of S protein into virus-like particles. J Gen Virol 97, 1853-1864.

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PLASMID PREPARATION

Plasmid resuspension

1. Quickly spin the tube containing the lyophilized plasmid to pellet the DNA.
2. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water.
3. 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.

GENERAL PROTOCOL

For a detailed protocol for producing SARS-CoV-2 spike (S)-pseudotyped lentiviral particles, please refer to the literature (*e.g. Crawford et al, 2020**). In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:
    - **InvivoGen's pLV-SARS2-S-d19** plasmid
    - Lentiviral backbone plasmid encoding a reporter protein (e.g. GFP or Luciferase)
    - Plasmid/s encoding the necessary virion packaging proteins
2. After ~48 hours, collect the (S)-pseudotyped lentiviral particles by harvesting and filtering the cell culture supernatant.
3. Determine the titre of the S-pseudotyped lentiviral particles using a permissive cell line that express the SARS-CoV-2 host receptor, hACE2, (e.g. InvivoGen's HEK-Blue™ hACE2 cells) in a relevent assay.

Crawford, K.H.D. et al. 2020. Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. Viruses 12. doi: 10.3390/v12050513

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent *E. coli*	gt116-11
pUNO1-hACE2	Expression vector	puno1-hace2
HEK-Blue™ hACE2 Cells	ACE2-expressing cell line	hkb-hace2

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