pLV-SpikeV1

Vector for lentiviral pseudotyping with SARS-CoV-2 Spike (G614-variant)

Catalog code: plv-spike-v1

https://www.invivogen.com/wuhan-spike-d614g-pseudotyping-vector

For research use only

Version 21D12-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pLV-SpikeV1** (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.

PLASMID FEATURES

- **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.

- Codon-optimized Spike ORF

pLV-SpikeV1 contains the Spike coding sequence from the Wuhan-Hu-1 SARS-CoV-2 G614-variant. This variant is characterized by the D614G mutation[1]. This signature mutation has globally replaced the original sequence and is present in all subsequent variants of SARS-CoV-2. Additionally, to improve expression of the S protein in pseudovirions, the gene is codon-optimized and the last 19 amino acids, which contain an endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed[2].

pLV-SpikeV1 includes the following sequence features:

- **S1 domain:** *D614G*
- **S1/S2 boundary:** *Functional furin cleavage site*

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 the proteolytic activity of host proteases such as furin and TMPRSS2[3].

Note: For more information visit: https://www.invivogen.com/sars2-spike

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

APPLICATION

pLV-SpikeV1 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (G614-variant). 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-SpikeV1. The transfected cells produce SARS-CoV-2 Spike-pseudotyped lentiviral particles, which can then be used to infect permissive cells.

GENERAL PROTOCOL

For a detailed protocol for producing SARS-CoV-2 spike (S)-pseudotyped lentiviral particles, please refer to the literature[4]. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:
   - **InvivoGen's pLV-SpikeV1** plasmid
   - Lentiviral backbone plasmid encoding a reporter protein (e.g. GFP or Luciferase)
   - Plasmid/s encoding the neccessary 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 (e.g. InvivoGen's HEK-Blue™ hACE2 cells) in a relevent assay.

PLASMID PREPARATION

- **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.

REFERENCES

1. Korber B. *et al.*, 2020. Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell. 182:1-16. **2. Johnson, M.C. *et al.* 2020.** Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. J Virol 94. **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. 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.

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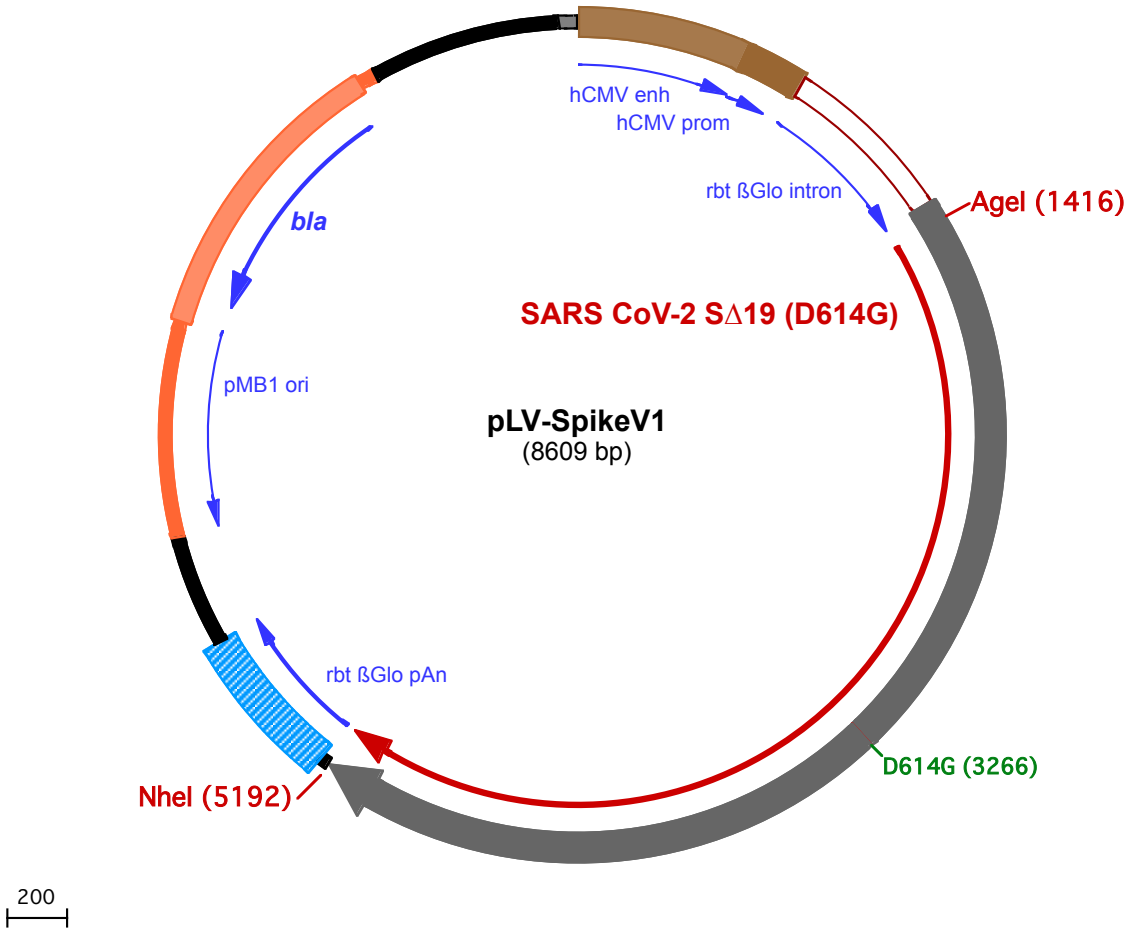
RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent *E. coli*	gt116-11
COVID-19 Product Range		
HEK-Blue™ hACE2 Cells	Cell line	hkb-hace2
A549-hACE2-TMPRSS2 Cells	Cell Line	a549-hace2-tpsa
pUNO1-hACE2	Expression vector	puno1-hace2
pUNO1-hTMPRSS2a	Expression vector	puno1-htp2a
Anti-CoV2RBD-c1-hIgG1	Recombinant Antibody	cov2rbdc1-mab1

For a complete list of InvivoGen's COVID-19 related products visit: https://www.invivogen.com/covid-19


hCMV enh
hCMV prom
rbt ßGlo intron
AgeI (1416)
bla
SARS CoV-2 SΔ19 (D614G)
pMB1 ori
pLV-SpikeV1
(8609 bp)
rbt ßGlo pAn
NheI (5192)
D614G (3266)
200


AgeI (1416)

D614G (3266)

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