pLV-SpikeV2

Expression vector for lentiviral pseudotyping with SARS-CoV-2 U.K. variant (B.1.1.7 lineage) Spike

Catalog code: plv-spike-v2

https://www.invivogen.com/uk-b117-spike-pseudotyping-vector

For research use only

Version 21D13-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pLV-SpikeV2** (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-SpikeV2 contains the Spike coding sequence from the United Kingdom (U.K.) SARS-CoV-2 variant (B.1.1.7 lineage). This variant is characterized by a number of deletions (del) and mutations within the Spike coding sequence (*see below*)[3]. Additionally, to improve expression of the S protein in pseudovirions, the gene is codon-optimized and the last 19 amino acids, which contain a endoplasmic reticulum (ER)-retention motif (KxHxx), have been removed[4,5].

pLV-SpikeV2 includes the following sequence features:

- **S1 domain:** *Del-H69-V70, Del-Y144, A570D, D614G, and P681H*
- **RBD:** *N501Y*
- **S1/S2 boundary:** *Functional furin cleavage site*
- **S2 domain:** *T716I, S982A, and D1118H*

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-SpikeV2 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (U.K. 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-SpikeV2. 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-SpikeV2** 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

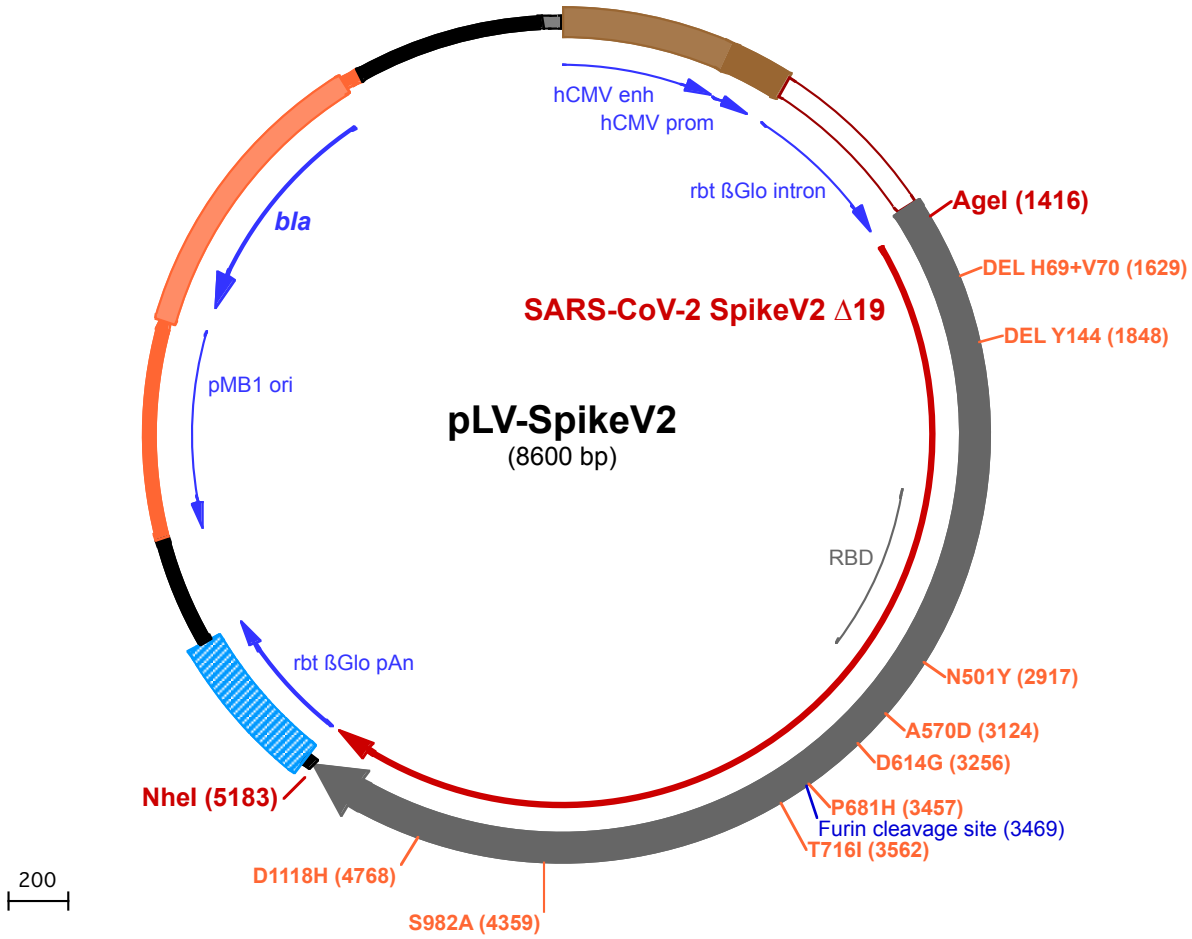
1. Shen, X. *et al.* 2021. SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral Spike vaccines. Cell Host and Microbe, doi: 10.1016/j.chom.2021.03.002. **2. Johnson, M.C. *et al.* 2020.** Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. J 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.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

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)
DEL H69+V70 (1629)
DEL Y144 (1848)
SARS-CoV-2 SpikeV2 Δ19
bla
pMB1 ori
pLV-SpikeV2
(8600 bp)
RBD
rbt ßGlo pAn
N501Y (2917)
A570D (3124)
D614G (3256)
P681H (3457)
Furin cleavage site (3469)
T716I (3562)
NheI (5183)
D1118H (4768)
S982A (4359)
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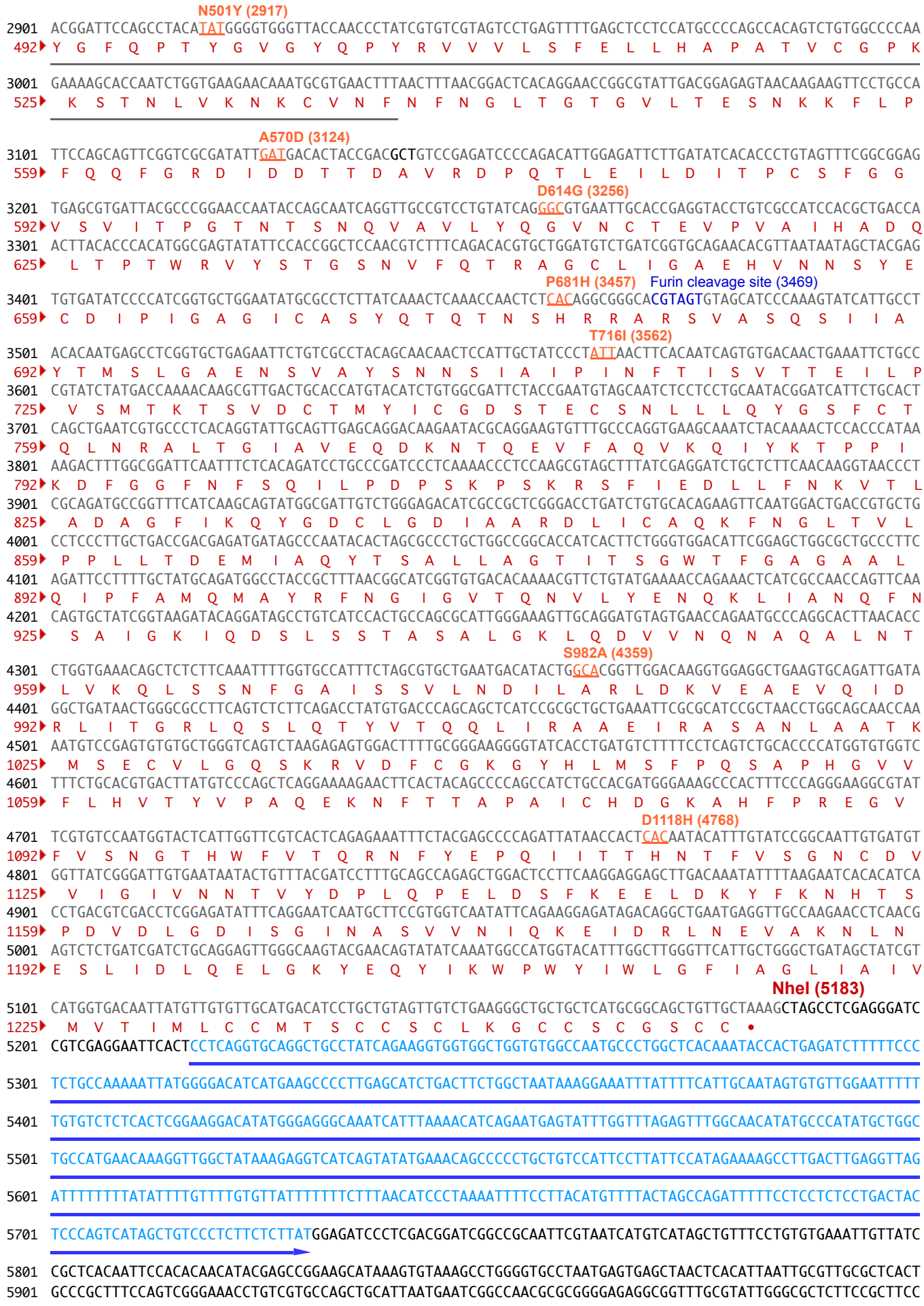
AgeI (1416)
DEL H69+V70 (1629)
DEL Y144 (1848)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Furin cleavage site (3469)
T716I (3562)
S982A (4359)
D1118H (4768)
NheI (5183)


6001 TCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACG
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287 • W H K I L S A G I E A I Q R N R E D M T A Q S
7001 TCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATC
263 G T T Y I V V I R S P K G D P G L A A I I G R S G R E G A G S K D
7101 AGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTA
230 A I F W G A P L A S R L L P G A V K D A E M W D I L Q Q R S A L T
7201 AGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTT
196 L L E G T L L K R L T T A M A V P M T T D R E D N P I A E N L E P E
7301 CCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGT
163 W R D L R T V H D G M N H L F A T L E K P G G I T T L L L N A A T
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130 N D S M T I A A S C L E R V T M G D T L H K E T V P S Y E V L D N
7501 TGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAA
96 Q S Y H I R R G L Q E Q G A D I R S L V A G C L L V K F T S M M P F
7601 AACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTAC
63 R E E P R F S E L I K G S N L D L E I Y G V R A G L Q D E A D K V
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