

pLV-SpikeV7

Vector for lentiviral pseudotyping with SARS-CoV-2 Kappa variant (B.1.617.1 lineage) Spike

Catalog code: plv-spike-v7

<https://www.invivogen.com/ind-b1617-spike-pseudotyping-vector>

For research use only

Version 21F25-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pLV-SpikeV7 (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-SpikeV7 contains the Spike coding sequence from the Kappa SARS-CoV-2 variant (B.1.617.1 lineage), first identified in India. This variant is characterized by a number of mutations within the the Spike coding sequence (*see below*)¹. 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 removed².

pLV-SpikeV7 includes the following sequence features:

- **S1 domain:** G142D, E154K, D614G, P681R
- **RBD:** L452R, E484Q
- **S1/S2 boundary:** Functional furin cleavage site
- **S2 domain:** Q1071H

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 TMPRSS2³.

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-SpikeV7 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (Kappa 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-SpikeV7. 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 literature⁴. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:

- **InvivoGen's pLV-SpikeV7** 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 (e.g. InvivoGen's **HEK-Blue™ hACE2 cells**) in a relevant 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. Liu, C. *et al.* 2021. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell, doi:10.1016/j.cell.2021.06.020.
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

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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>

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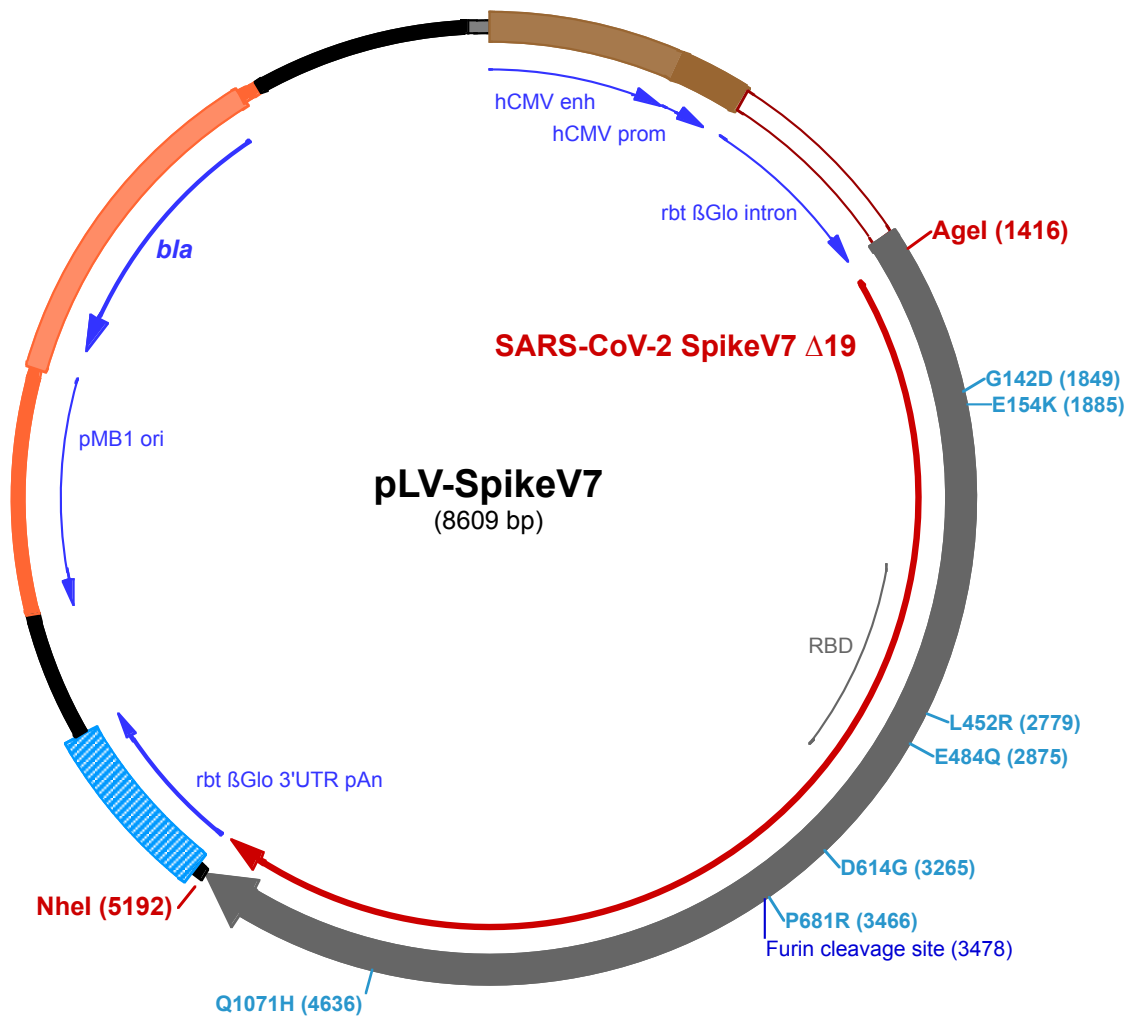
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1 GAGCTTGGCCCATTCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACGCCATGTTGACATTGATTATTGACTA
101 GTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTTACATAACTACGGTAAATGGCCCGCTGGCTGACCGCC
201 CAACGACCCCGCCCATTCGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA
301 ACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACA
401 TGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATA
501 GCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTCGACGTCAATGGGAGTTTGTGTTGGCACCAAATCAACGGGACTTTCCAAAATGTCGTAAC
601 AACTCCGCCCATTCGACGCAAATGGGCGGTAGGCGGTACGGTGGGAGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCC
701 ATCCACGCTGTTTTGACCTCCATAGAAGACACCGGACCGATCCAGCTCCGGTGCACCGATCCTGAGAACTCAGGgtgagtttggggacccttgattg
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Agel (1416)

1401 ATTCTCGACGGATCCACCGGTCAACATGTTTGTGTTCTTGGTGTGCTTCCACTGGTCAGTCCCAATGCGTTAATCTCACACCCGAACCTCAACTCCC
1501 ACCCGCATATACAAATTCCTTACCAGAGGAGTGTACTATCCTGACAAAGTGTTCGGTCAAGTGTCTCCACTCTACTCAGGACCTCTTTCTGCCTTTC
1601 TTTTCTAACGTTACATGGTTTCATGCAATCCATGTGTCTGGGACAAACGGCACAAACGCTTCGACAAACCTGATTGCCATTCAATGATGGGGTGTACT
1701 TTGCCTCCACAGAGAAATCCAACATCATTGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGTATGATGTTAAACAACGCCACAAA
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G142D (1849)

1801 V V I K V C E F Q F C N D P F L D V Y Y H K N N K S W M K S E F R
1901 GTCTACAGCAGCGCAAACACTGCACCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGAAACAGGGAAACTCAAGAACCTGAGAGAGT
2001 TTGTCTTTAAGAACATCGACGGCTATTTAAGATCTATAGTAAGCATACGCCATCAACCTGGTAAGGGATCTCCCCAGGGCTTTTCAGCCCTGGAACC
2101 TTTGGTTGACTTGCTATTGGTATCAATATCACCAGATTTAGACCCTTCTGGCATTGCATCGGTCTTATCTTACTCCAGGTATTCTCTCCGGGTGG
2201 ACTGCCGCGCCGCTGCCTACTATGTGCGGTATCTGCAACCAAGAAGTTCCTGCTCAAGTACAACGAAAACGGCACTATTACGGATGCTGTTGATTGTG
2301 CCCTGGACCTCTGTCTGAGACTAAATGCACCTCAAGAGCTTTACCGTTGAGAAGGGGATTTACCAAACAGTAATTTCCGGGTCCAACCCACCGAAAG
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E154K (1885)

2401 I V R F P N I T N L C P F G E V F N A T R F A S V Y A W N R K R I
2501 TCCAATTGTGTCGCTGATTACTCCGTGCTGTACAATCCGCCTCTTTCTCAACCTTCAAGTGTATGGCGTTTACCTACCAAACCTAACGACCTGTGCT
2601 TCACTAATGTGTATGCCGACTCTTTTGTGATACGAGGCGATGAAGTGAAGCAGATTGCACCAGGGCAGACCGGCAAATTTGCCGACTACAACATAAGCT
2701 TCCAGATGACTTTACCGGATGTGTTATTGCATGGAACCTCAAACAATCTGGATTCCAAGTGGGTGGCAACTATAACTACCGCTATAGACTGTTCCAGGAAA

L452R (2779)

2701 P D D F T G C V I A W N S N N L D S K V G G N Y N Y R Y R L F R K

E484Q (2875)

2801 TCCAACCTGAAACATTTCGAGCGAGATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGCAAGGGTTCAACTGCTACTTCCCAT
2801 S N L K P F E R D I S T E I Y Q A G S T P C N G V Q G F N C Y F P

2901 TGCAGAGTTACGGATTCCAGCCTACAAACGGGGTGGGTTACCAACCCTATCGTGTCTAGTCTGAGTTTTGAGCTCCTCCATGCCCCAGCCACAGTCTG
492▶ L Q S Y G F Q P T N G V G Y Q P Y R V V V L S F E L L H A P A T V C

3001 TGGCCCCAAGAAAAGCACCAATCTGGTGAAGAACAATGCGTGAACITTAACITTAACGGACTCACAGGAACCGGCGTATTGACGGAGAGTAACAAGAAG
525▶ G P K K S T N L V K N K C V N F N F N G L T G T G V L T E S N K K

3101 TTCCTGCCATTCCAGCAGTTCGGTGCAGATATTGCCGACACTACCGACGCTGTCCGAGATCCCAGACATTGGAGATTCTTGATATCACACCTGTAGTT
559▶ F L P F Q Q F G R D I A D T T D A V R D P Q T L E I L D I T P C S
D614G (3265)

3201 TCGGCGGAGTGAGCGTGATTACGCCCGGAACCAATACCAGCAATCAGGTTGCCGCTGTATCAGGGCGTGAATTGCACCGAGGTACCTGTCGCATCCA
592▶ F G G V S V I T P G T N T S N Q V A V L Y Q G V N C T E V P V A I H

3301 CGCTGACCAACTTACACCCACATGGCGAGTATATTCCACCGGCTCCAACGCTTTTCAGACAGTGCTGGATGCTGATCGGTGCGAACAACGTTAATAAT
625▶ A D Q L T P T W R V Y S T G S N V F Q T R A G C L I G A E H V N N
P681R (3466) Furin cleavage site (3478)

3401 AGCTACGAGTGTGATATCCCCATCGGTGCTGGAATATGCGCCTTTATCAAACCTCAAACCAACTCTCGTAGGGCGGACAGTAGTGTAGCATCCCAAAGTA
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3501 TCATTGCCTACACAATGAGCCTCGGTGCTGAGAATTTGTGCGCTACAGCAACAACCTCATTGCTATCCCTACTAACTTACAATCAGTGTGACAACTGA
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3601 AATTCTGCCCGTATCTATGACCAAAAACAGCGTTGACTGCACCATGTACATCTGTGGCGATTCTACCGAATGTAGCAATCTCCTCCTGCAATACGGATCA
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3701 TTCTGCACTCAGTGAATCGTGCCTCACAGGATTGCAGTTGAGCAGGACAAGAATACCGAGGAAGTGTTCGCCAGGTGAAGCAAACTACAAAACCTC
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3801 CACCCATAAAAAGACTTTGGCGGATTCAATTTCTCACAGATCCTGCCGATCCCTCAAACCCCTCAAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAA
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3901 GGTAAACCTCGCAGATGCCGTTTCATCAAGCAGTATGGCATTGTCTGGGAGACATCGCCGCTCGGACCTGATCTGTGCACAGAAGTTCAATGGACTG
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4001 ACCGTGCTGCCTCCCTTGTGACCGACGAGATGATAGCCCAATACACTAGCGCCTGCTGGCCGGCACCATCACTTCTGGGTGGACATTGGAGCTGGCG
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892▶ A A L Q I P F A M Q M A Y R F N G I G V T Q N V L Y E N Q K L I A N

4201 CCAGTTCAACAGTGTATCGGTAAGATACAGGATAGCTGTCTCACTGCCAGCGCATTGGGAAAGTTGCAGGATGTAGTGAACCAGAATGCCAGGCA
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4301 CTTAAACCCCTGGTGAACAGCTCTCTCAAATTTTGGTGCATTTCTAGCGTGTGAATGACATACTGAGCCGTTGGACAAGGTGGAGGCTGAAGTGC
959▶ L N T L V K Q L S S N F G A I S S V L N D I L S R L D K V E A E V

4401 AGATTGATAGGCTGATAACTGGGCGCCTTCACTCTCTCAGACCTATGTGACCAGCAGCTCATCCGCGTGTGAAATTCGCGCATCCGCTAACCTGGC
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Q1071H (4636)

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1125▶ C D V V I G I V N N T V Y D P L Q P E L D S F K E E L D K Y F K N

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NheI (5192)

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1225▶ A I V M V T I M L C C M T S C C S C L K G C C S C G S C C •

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5401 GGAATTTTTTGTGCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCA

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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
7001 TTGCTGACTCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCAAGTGTGCAATGATACCGCGAGACCCAGCTCACC GGCTCC
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199 A L T 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
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