

pLV-SpikeV3

Vector for lentiviral pseudotyping with SARS-CoV-2 South African variant (B.1.351 lineage) Spike

Catalog code: plv-spike-v3

<https://www.invivogen.com/sa-b1351-spike-pseudotyping-vector>

For research use only

Version 21Z05-ED

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pLV-SpikeV3 (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-SpikeV3 contains the Spike coding sequence from the South African (S.A.) SARS-CoV-2 variant (B.1.351 lineage). This variant is characterized by a number of deletions (del) and mutations within the 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-SpikeV3 includes the following sequence features:

- **S1 domain:** L18F, D80A, D215G, Del-242-244, D614G
- **RBD:** K417N, E484K, N501Y
- **S1/S2 boundary:** Functional furin cleavage site
- **S2 domain:** A701V

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-SpikeV3 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (South African 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-SpikeV3. 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-SpikeV3** 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. Tegally, H. *et al.* 2020. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *Medrxiv* doi:10.1101/2020.12.21.20248640v1. 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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RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent <i>E. coli</i>	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>

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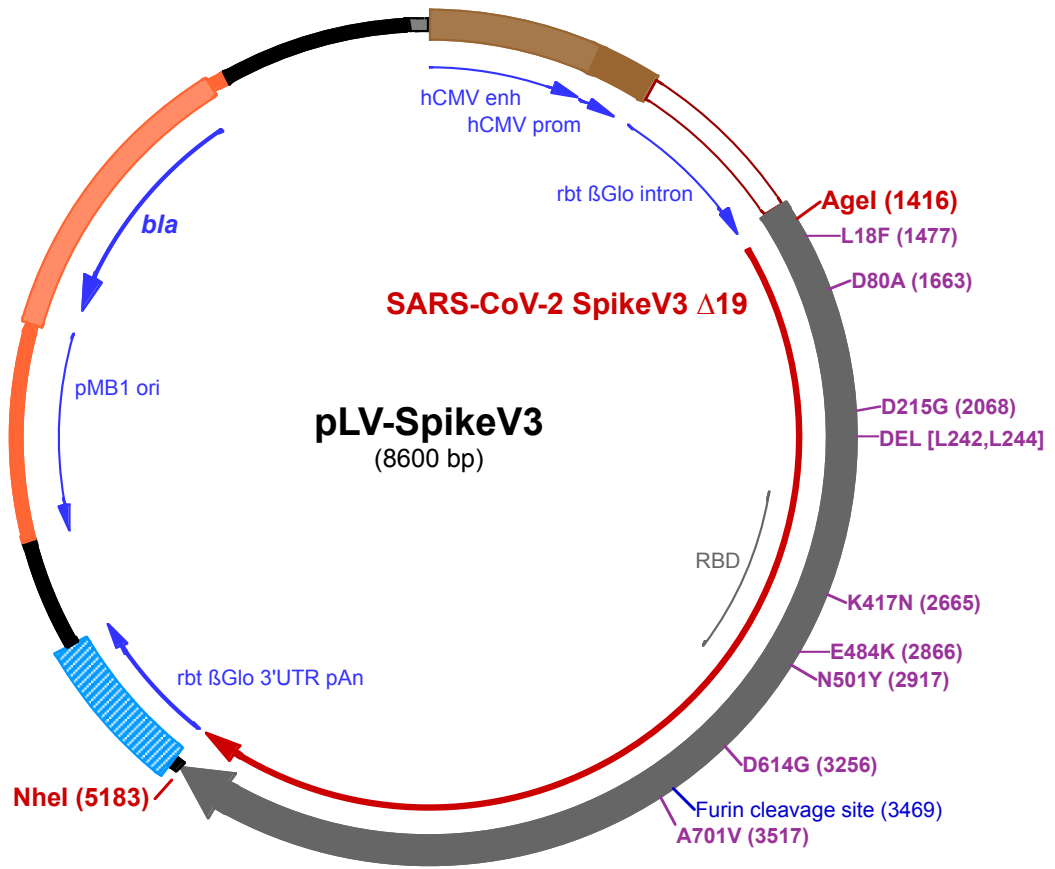
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200

1 GAGCTTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACGCCATGTTGACATTGATTATTGACTA
101 GTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCC
201 CAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA
301 ACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACA
401 TGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATA
501 GCGGTTTGACTCACGGGGATTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTGGCACAAAATCAACGGGACTTTCCAAAATGTCGTAAC
601 AACTCCGCCCATGACGCAAATGGGCGGTAGCGGTGTACGGTGGGAGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCC
701 ATCCACGCTGTTTGGACTCCATAGAAGACACCGGGACCGATCCAGCTCCGGTGCACCGATCCTGAGAACTCAGGgtgagtttggggacccttgattg
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1201 attggtagaacaactacaccctggtcatcatcctgcctttctctttatggttacaatgatatacactgtttgagatgaggataaaaactctgagtcca
1301 aaccgggcccctctgtaacatggtcatgcctttctctttctacagCTCCTGGCAACGTGCTGTTGTTGTGCTGTCTCATATTTGGCAAAGA

Agel (1416)

L18F (1477)

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1501 ACCCGCATATACAAATTCCTTACCAGAGGAGTACTATCCTGACAAAGTGTTCGGTCAAGTGTCTCCACTCTACTCAGGACCTCTTTCTGCCTTTC
25▶ P A Y T N S F T R G V Y Y P D K V F R S S V L H S T Q D L F L P F
1601 TTTCTAACGTTACATGGTTTCATGCAATCCATGTGTCTGGGACAAACGGCACAAACGCTTCCGTAACCTGTATTGCCATTCAATGATGGGGTGTACT
59▶ F S N V T W F H A I H V S G T N G T K R F A N P V L P F N D G V Y
1701 TTGCTCCACAGAGAAATCCAACATCATTGAGGATGGATTTTCGGGACTACTCTGGACTCAAAGACACAGAGCCTGCTGATCGTTAAACAACGCCACAAA
92▶ F A S T E K S N I I R G W I F G T T L D S K T Q S L L I V N N A T N
1801 CGTTGTCATCAAAGTGTGCGAATTCAGTTCGCAATGATCCCTTCCGGGAGTGTACTATACAAGAATAACAAGTCTGGATGGAGAGCGAATTTCCGG
125▶ V V I K V C E F Q F C N D P F L G V Y Y H K N N K S W M E S E F R
1901 GTCTACAGCAGCGCAAACAATGACCTTCGAGTACGTGAGTCAACCTTTCTGATGGACCTGGAAGGAAACAGGAAACTCAAGAACCTGAGAGAGT
159▶ V Y S S A N N C T F E Y V S Q P F L M D L E G K Q G N F K N L R E

D80A (1663)

D215G (2068)

2001 TTGTCTTAAAGAACATCGACGGCTATTTAAGATCTATAGTAAGCATACGCCATCAACCTGGTAAGGGGCTTCCCCAGGGCTTTTCAGCCCTGGAACC
192▶ F V F K N I D G Y F K I Y S K H T P I N L V R G L P Q G F S A L E P
2101 TTTGGTTGACTTGCTATTGGTATCAATATCACCAGATTTAGACCCTTTCATCGGTCTTATCTTACTCCAGGTGATTCCTCCTCCGGTGGACTGCCGGC
225▶ L V D L P I G I N I T R F Q T L H R S Y L T P G D S S S G W T A G
2201 GCCGCTGCCTACTATGTCGGCTATCTGCAACCAAGAAGCTTCTGCTCAAGTACAACGAAAACGGCACTATTACGGATGCTGTTGATTGTGCCTGGACC
259▶ A A A Y Y V G Y L Q P R T F L L K Y N E N G T I T D A V D C A L D
2301 CTCTGCTGAGACTAAATGCACCCTCAAGAGCTTACCCTGAGAAGGGGATTACCAAACAGTAATTTCCGGTCCAACCCACCGAAAGCATTGTGCG
292▶ P L S E T K C T L K S F T V E K G I Y Q T S N F R V Q P T E S I V R

DEL [L242,L244]

2401 GTTCCCAAATATACCAATCTGTGTCCTTTGGCGAAGTGTCAATGCTACAAGTTTGTCTGTGTACGCATGGAATAGGAAACGCATCTCCAATTGT
325▶ 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 S N C
2501 GTCGCTGATTACTCCGTGCTGTACAATTCGGCCTTTCTCAACCTTCAAGTGTATGGGTTTACCTACCAAACCTAACGACCTGTGCTTCACTAATG
359▶ V A D Y S V L Y N S A S F S T F K C Y G V S P T K L N D L C F T N

K417N (2665)

2601 TGTATGCCGACTTTTTGTGATACGAGCGATGAAGTGAAGACAGATTGACACAGGGCAGACCGGCAATTTGCGGACTACAACATAAGCTTCCAGATGA
392▶ V Y A D S F V I R G D E V R Q I A P G Q T G N I A D Y N Y K L P D D
2701 CTTTACCGGATGTGTTATTGCATGGAACCTCAACAATCTGGATTCCAAGTGGGTGGCAACTATAACTACCTGTATAGACTGTTCCAGGAAATCCAACCTG
425▶ F T G C V I A W N S N N L D S K V G G N Y N Y L Y R L F R K S N L

E484K (2866)

2801 AAACCATTCGAGCGAGATATAAGCACAGAAATCTACCAGGCTGGAAGTACGCCCTGCAACGGCGTGA^{AAA}GGGTTCAACTGCTACTTCCCATTGCAGAGTT
459▶ K P F E R D I S T E I Y Q A G S T P C N G V K G F N C Y F P L Q S

N501Y (2917)

2901 ACGGATTCCAGCCTACA^{TAT}GGGGTGGGTTACCAACCCATCGTGTGCTAGTCTGAGTTTTGAGCTCCTCCATGCCCCAGCCACAGTCTGTGGCCCCAA
492▶ Y G F Q P T Y G V G Y Q P Y R V V V L S F E L L H A P A T V C G P K

3001 GAAAAGCACCAATCTGGTGAAGAACAATGCGTGAAC^{TTA}CTTTAACGGACTCACAGGAACCGCGTATTGACGGAGAGTAACAAGAAGTTCC^IGCCA
525▶ 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 F L P

3101 TTCCAGCAGTTGCGTGCAGATATTGCCGACACTACCAGC^{GCT}GTCCGAGATCCCAGACATTGGAGATTCTTGATATCACACCCTGTAGTTTCGGCGGAG
559▶ 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 F G G

D614G (3256)

3201 TGAGCGTGATTACGCCCGGAACCAATACAGCAATCAGGTTGCCGTCCTGTATCAG^{GCT}GTGAATTGCACCGAGTACCTGTCCCATCCACGCTGACCA
592▶ 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 A D Q

3301 ACTTACACCCACATGGCGAGTATATTCCACCGCTCCAACGCTTTTACAGACAGTGGTGGATGCTGATCGGTGCAGAACACGTTAATAATAGCTACGAG
625▶ 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 S Y E

Furin cleavage site (3469)

3401 TGTGATATCCCCATCGGTGCTGGAATATGCGCCTTTATCAAACCTCAAACCACTCTCCTAGCGGGCA^{CGTAGT}GTAGCATCCCAAAGTATCATTGCCT
659▶ C D I P I G A G I C A S Y Q T Q T N S P R R A R S V A S Q S I I A

A701V (3517)

3501 ACACAATGAGCCTCGGT^{GTA}GAGAATTCTGTCGCCTACAGCAACAACCTCCATTGCTATCCCTACTA^{ACT}TACAATCAGTGTGACAACTGAAATTCTGCC
692▶ Y T M S L G V E N S V A Y S N N S I A I P T N F T I S V T T E I L P

3601 CGTATCTATGACCAAAACAAGCGTTGACTGCACCATGTACATCTGTGGCGATTCTACCGAATGTAGCAATCTCCTCCTGCAATACGGATCATTCTGCACT
725▶ V S M T K T S V D C T M Y I C G D S T E C S N L L L Q Y G S F C T

3701 CAGCTGAATCGTCCCTCACAGGATTGCGATTGAGCAGGACAAGAATACGCGAGGAAGTGTGGCCAGGTGAAGCAAATCTACAAAACCTCCACCCATAA
759▶ Q L N R A L T G I A V E Q D K N T Q E V F A Q V K Q I Y K T P P I

3801 AAGACTTTGGCGGATTCAATTTCTCACAGATCTGCCGATCCCTCAAACCCCTCAAAGCGTAGCTTTATCGAGGATCTGCTCTTCAACAAGTAACCT
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3901 CGCAGATGCCGTTTCATCAAGCAGTATGGCGATTGTCTGGGAGACATCGCCGCTCGGACCTGATCTGTGCACAGAAGTTCAATGGACTGACCGTGTG
825▶ A D A G F I K Q Y G D C L G D I A A R D L I C A Q K F N G L T V L

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892▶ 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 Q T F N

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925▶ S A I G K I Q D S L S S T A S A L G K L Q D V V N Q N A Q A L N T

4301 CTGGTGAAACAGCTCTCTTCAAATTTTGGTGCCATTCTAGCGTGTGAATGACATACTGAGCCGGTTGGACAAGGTGGAGGCTGAAGTGCAGATTGATA
959▶ 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 Q I D

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4501 AATGTCCGAGTGTGTGCTGGGTGACTAAGAGAGTGGACTTTTGGCGGAAGGGGTATCACTGATGTCTTTTCTCAGTCTGACCCCATGGTGTGGTC
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4801 GGTATCGGGATTGTGAATAACTGTTTACGATCCTTTGAGCCAGAGCTGGACTCCTTCAAGGAGGAGCTTGACAAATATTTAAGAATCACACATCA
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5001 AGTCTCTGATCGATCTGCAGGAGTTGGGCAAGTACGAACAGTATATCAAATGGCCATGGTACATTTGGCTTGGGTTCAATGCTGGGCTGATAGCTATCGT
1192▶ E S L I D L Q E L G K Y E Q Y I K W P W Y I W L G F I A G L I A I V

NheI (5183)

5101 CATGGTGACAATTATGTTGTGTTGCATGACATCCTGCTGAGTTGTCTGAAGGGCTGTGCTCATGCGGCAGCTGTTGCTAAAGCTAGCCTCGAGGGATC
1225▶ 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 •

5201 CGTCGAGGAATCACTCCTCAGGTGCAGGCTGCCTATCAGAAGGTGGTGGTGGTGGCCAAATGCCCTGGCTCACAATACCACTGAGATCTTTTTCCC

5301 TCTGCCAAAATTATGGGACATCATGAAGCCCCTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTT

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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
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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
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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
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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
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7701 TTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACCGGAAATGTTGAATACTCATACTCTTCTT
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