

pLV-SpikeV11

Vector for lentiviral pseudotyping with SARS-CoV-2 Omicron variant (B.1.1.529 lineage) Spike

Catalog code: plv-spike-v11

<https://www.invivogen.com/omicron-b11529-spike-pseudotyping-vector>

For research use only

Version 21K09-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pLV-SpikeV11 (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-SpikeV11 contains the Spike coding sequence from the Omicron SARS-CoV-2 variant (B.1.1.529 lineage), first identified in South Africa in late November 2021. This variant is characterized by a number of mutations and deletions within the the Spike coding sequence (see below)^{1,2}. 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 removed³.

pLV-SpikeV11 includes the following sequence features:

- **S1 domain:** TA67V, deletion (Δ)H69+V70, T95I, G142D, ΔV143-Y145, ΔN211, ins214EPE, T547K, D614G, H655Y, N679K, P681H
- **RBD:** G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H
- **S1/S2 boundary:** Functional furin cleavage site
- **S2 domain:** N764K, D796Y, N856K, Q954H, N969

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 TMPRSS2⁴.

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-SpikeV11 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (Omicron 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-SpikeV11. 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 literature⁵. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:
 - InvivoGen's pLV-SpikeV11 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 titer of the S-pseudotyped lentiviral particles using a permissive cell line that expresses 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. <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/>.
2. <https://outbreak.info/situation-reports> 3. Johnson, M.C. et al. 2020. Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. *J Virol* 94. 4. 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. 5. 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-cas-hlgG1	Recombinant Antibody	srbdc3-mab1

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