pLV-SpikeV10

Vector for lentiviral pseudotyping with SARS-CoV-2 Mu variant (B.1.621 lineage) Spike

Catalog code: plv-spike-v10

https://www.invivogen.com/mu-b1621-spike-pseudotyping-vector

For research use only

Version 21J25-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pLV-SpikeV10** (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-SpikeV10 contains the Spike coding sequence from the Mu SARS-CoV-2 variant (B.1.621 lineage), first identified in Colombia. 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 removed[3].

pLV-SpikeV10 includes the following sequence features:

- **S1 domain:** *T95I, Y144T, Y145S, ins145N, D614G, P681H*
- **RBD:** *R346K, E484K, N501Y*
- **S1/S2 boundary:** *Functional furin cleavage site*
- **S2 domain:** *D950N*

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[4].

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-SpikeV10 has been designed for pseudotyping lentiviral particles with the SARS-CoV-2 Spike protein (Mu 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-SpikeV10. 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[5]. In summary,

1. Co-transfect HEK293 cells with the plasmids required for lentiviral production. These include:
   - **InvivoGen's pLV-SpikeV10** 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 titer 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. 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.

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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-hIgG1	Recombinant Antibody	srbdc3-mab1

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