sgRNA for human PD-L1 (Puro) Lentifect™ Purified Lentiviral Particles

Cat. No. LP624-050 (Old Cat. No. LPP-HCP001057-LvSG03-15-10-h-50)

Ready-to-use purified lentiviral particles, used together with Cas9 nuclease lentiviral particles (See Cat No. followed) for effective human PD-L1 gene knock out in a variety of mammalian cells including difficult-to-transfect, primary, stem and non-dividing cells as well as in vivo use for transgenic animals.

PD-L1 gene knock out validated in HEK293 cells

Description

Gene: sgRNA (target in human PD-L1 gene)
Promoter: U6
Tag: N/A
Reporter: mCherry
Resistance marker: Puromycin
Additional note: N/A

GeneCopoeia Lentifect™ Lentiviral Particles are produced from a standardized protocol using purified plasmid DNA and the proprietary reagents, EndoFectin™ Lenti (for transfection) and TiterBoost™ solution. The protocol uses a third generation self-inactivating packaging system meeting BioSafety Level 2 requirements.

Contents and storage

Provided as 1 vial of 50 µl purified sgRNA for human PD-L1 lentiviral particles with titers of ~1 x 10^8 TU/ml.

Lentiviral particles are shipped on dry ice and must be stored at -80°C immediately upon receipt. Avoid repeated freeze-thaw cycles as this will reduce titers.

Suggested Cas9 Nuclease Lentiviral Particles from GeneCopoeia

The product can be combined with any of the following Ready-to-use Cas9 nuclease lentiviral particles for co-transduction or Cas9 sgRNA sequential transduction:

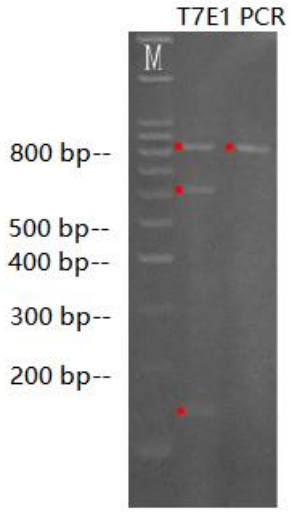
LP601-100
LP602-100
LP604-100
LP605-100

Quality control

The lentiviral expression construct was validated by full-length sequencing, restriction enzyme digestion and PCR-size validation using gene-specific and vector-specific primers. Product is confirmed free of bacteria, fungi, common *Mycoplasma* contamination and T7 E1 Assay.

T7 Endonuclease I (T7 E1) Assay

PD-L1 Site T7 E1 Assay
sgRNA targeting to PD-L1 gene was transduced into HEK293/Cas9 Stable Cell Line by transduction. PD-L1 gene was cut by CAS9 expressed inside the cells and repaired through NHEJ with mutation. An 823 bp PD-L1 gene fragment from PCR was then tested by T7 Endonuclease I (T7 E1) Assay. The T7 E1 cleavage will results in two additional bands: one ~672 bp and the other ~151 bp.



Viral titer

The transduction unit (TU or IFU) of the lentiviral particles was estimated using the formula- 1TU=100 copies of viral genomic RNA. The physical copy numbers of the viral genomic RNA was determined using qRT-PCR. The customer should test the transduction at MOI=0.3, 1, 3, 5, 10 for their specific cell lines in order to get the best transduction efficiency.

Overview of production

The lentiviral particles were generated by following a standardized protocol using highly purified plasmids and EndoFectin-Lenti™ and TiterBoost™ reagents.

The lentiviral transfer vector was co-transfected into 293Ta cells (GeneCopoeia Cat. No. LT008) with the Lenti-Pac™ HIV Packaging Mix (GeneCopoeia Cat. No. LT001). Lentivirus-containing supernatants were harvested 48 hours after transfection and stored at -80°C.

User protocol

The large insertion size of cas9 expression cassette leads to the sharp titer decline of Cas9 lentiviral particles. To get effective desired genome editing, we strongly suggest antibiotic selection with indicate resistance gene to collect cells genomically integrated cas9 after transduction.

User manual

Please contact GeneCopoeia for a copy or download at:
https://www.genecopoeia.com/wp-content/uploads/2018/03/Lentivirus-protocol-GeneCopoeia.pdf

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