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Data Sheet

PD-L1 CRISPR/Cas9 Lentivirus (Non-Integrating)

Catalog #: 78064

Description

The binding of Programmed Cell Death Protein 1 (PD-1), a receptor expressed on activated T-cells, to its ligands, PD-L1 and PD-L2, negatively regulates immune responses. The PD-1 ligands are found on most cancers, and the PD-1:PD-L1/2 interaction inhibits T-cell activity and allows cancer cells to escape immune surveillance. The PD-1:PD-L1/2 pathway is also involved in regulating autoimmune responses, making these proteins promising therapeutic targets for a number of cancers, as well as multiple sclerosis, arthritis, lupus, and type I diabetes.

The PD-L1 CRISPR Lentiviruses are replication incompetent, HIV-based, VSV-G pseudo-typed lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a CRISPR/Cas9 gene driven by an EF1a promoter, along with 4 sgRNA (single guide RNA) targeting human PD-L1 (Programmed Cell Death 1 Ligand 1, CD274, B7 homolog 1 (B7-H1), GenBank accession #NM_021893) driven by a U6 promoter (Figures 1 and 2).

Note: unlike Human PD-L1 CRISPR/Cas9 Lentivirus (Integrating) (BPS Bioscience, #78057), the Human PD-L1 CRISPR/Cas9 Lentivirus (Non-Integrating) is made with a mutated Integrase, resulting in only transient expression of the Cas9 and PD-L1-targeting sgRNA. While this may minimize potential off-targeting risks due to either prolonged expression or integration of the Cas9, puromycin selection should not be used for more than 48 hours post-transduction, which may lower knockout efficiency.

Application

1. Transient knock-down of PD-L1 in a target cell pool.
2. Generation of a stable PD-L1 knock-out cell line following limited dilution.

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 μ l x 2) of lentivirus at a titer $\geq 1 \times 10^6$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

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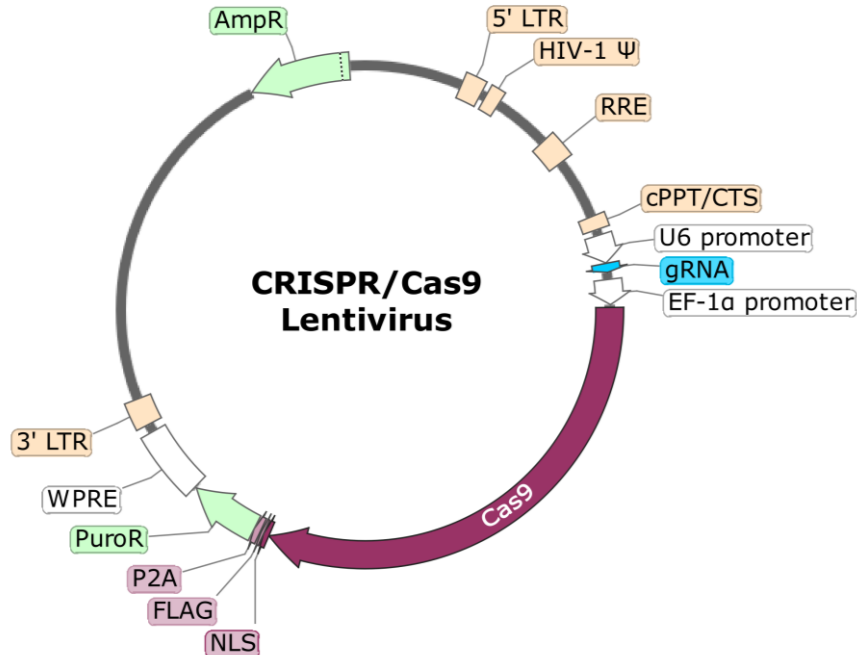


Figure 1. Schematic of the Lenti-vector used to generate the PD-L1 CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
PD-L1	CD274-1-1	GGTCCCAAGGACCTATATG
PD-L1	CD274-1-2	ACTGCTTGTCAGATGACTT
PD-L1	CD274-1-3	GCATAGTAGCTACAGACAGA
PD-L1	CD274-1-4	ACATGTCAGTTCATGTTTCAG

Figure 2. List of sgRNA Sequences in the PD-L1 CRISPR/Cas9 Lentivirus.

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the lentiviruses at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells. Although the pseudotyped lentiviruses are replication-incompetent, they do require the use of a Biosafety Level 2 facility. BPS recommends following all federal, state, local, and institutional regulations and using all appropriate safety precautions.

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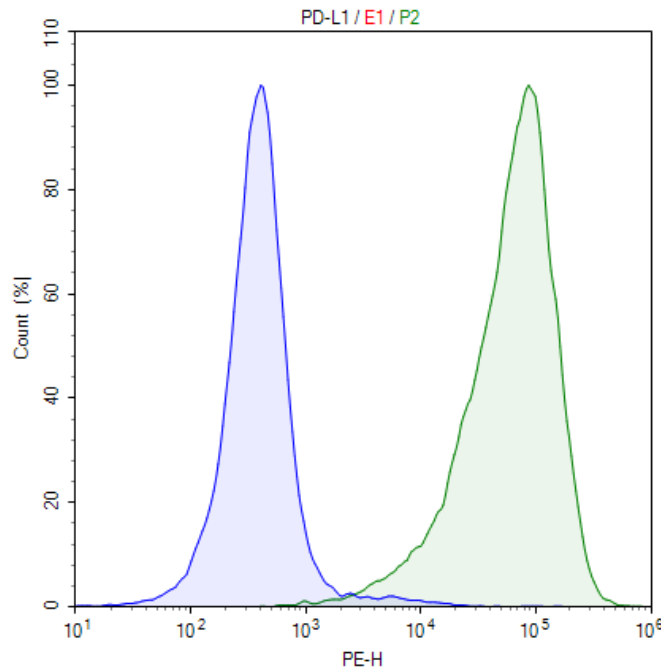


Figure 3. Knock-down of PD-L1 in PD-L1 Over-expressing CHO cells.

PD-L1 Over-expressing CHO cells (BPS Bioscience, #60543) were transduced with 5,000,000 TU/well of PD-L1 CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE-labeled anti-human PD-L1 antibody (BioLegend, #329705) and analyzed by FACS. Parental PD-L1 CHO cells are shown in green, and the transduced cells are shown in blue.

Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
PD-L1 CRISPR/Cas9 Lentivirus (Integrating)	78057	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Integrating)	78052	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78059	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2
Cas9, His-tag (<i>S. pyogenes</i>)	100206-1	50 µg
PD-L1 / TCR Activator - CHO Recombinant Cell Line	60536	2 vials
PD-L1 - CHO Recombinant Cell Line	60543	2 vials
Anti-PD-L1 (CD274) Neutralizing Antibody	71213	100 µg
PD-1 - HEK293 Recombinant Cell Line	60680	2 vials
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials

Notes

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

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