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efData Sheet

PD-L1 CRISPR/Cas9 Lentivirus (Integrating)

Catalog #: 78057

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).

The integrating lentivirus integrates randomly into the cell's genome to express both the Cas9 and sgRNA. Puromycin selection increases the knockout efficiency by forcing high expression levels of both Cas9 and the sgRNA, and can be used with the integrating lentivirus to quickly and easily achieve high knockdown efficiencies in a cell pool. Efficiencies also depend on the cell type and the gene of interest.

Application

1. Transient knock-down of PD-L1 in target cells.
2. Generation of a stable PD-L1 knock-out cell line following puromycin selection.

Formulation

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

Titer

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

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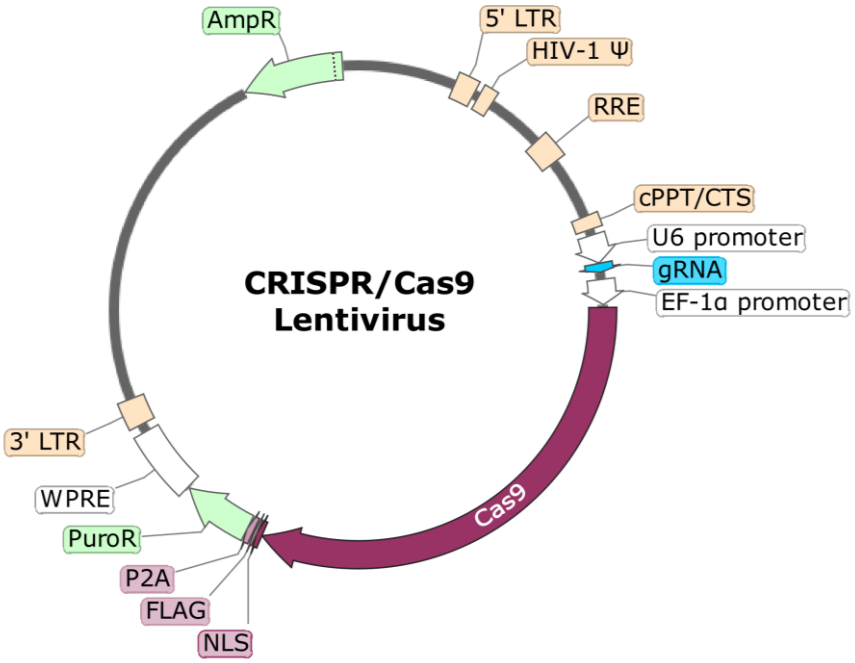

Figure 1. Schematic of the Lenti-vector used to generate the PD-L1 CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
PD-L1	CD274-1-1	GGTTCCCAAGGACCTATATG
PD-L1	CD274-1-2	ACTGCTTGTCCAGATGACTT
PD-L1	CD274-1-3	GCATAGTAGCTACAGACAGA
PD-L1	CD274-1-4	ACATGTCAGTTCATGTTCAG

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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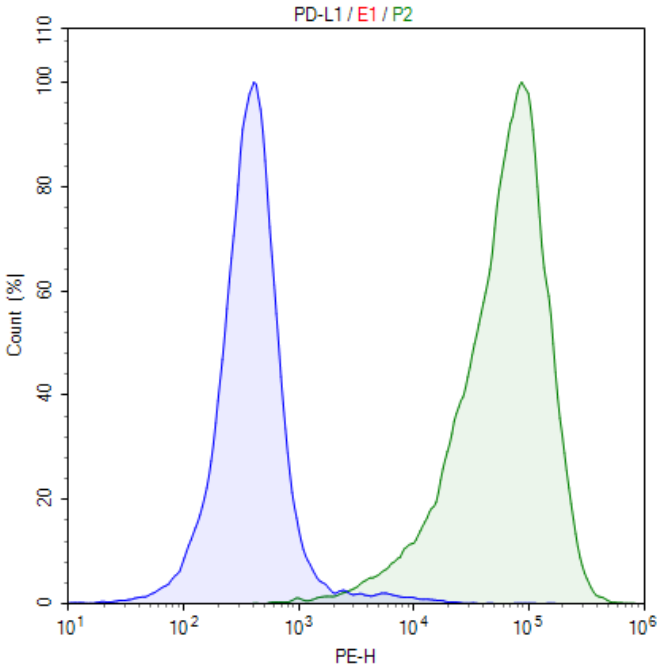

Figure 3. Knock-down of PD-L1 in PD-L1 Over-expressing CHO cells.
PD-L1 Over-expressing CHO cells (BPS Bioscience, #60543) were transduced with 5,000,000 TU/well of PD-L1 CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE-labeled anti-human PD-L1 antibody (BioLegend, #329705) and analyzed by FACS. Parental PD-L1 CHO cells are shown in green, and the transduced cells are shown in blue.

Related Products

Product	Cat. #	Size
PD-L1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78064	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78059	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Integrating)	78052	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 (S. pyogenes)	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.