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. PD-1 ligands are found on most cancer cells, and the PD-1:PD-L1/2 interaction inhibits T cell activity and enables 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 cancer types, as well as multiple sclerosis, arthritis, lupus, and type I diabetes.

The PD-1 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 pseudovirus particles contain a CRISPR/Cas9 gene driven by an EF1A promoter, along with 4 sgRNA (single guide RNA) targeting human PD-1 (Programmed Cell Death 1, PDCD1, CD279, GenBank Accession #NM_005018) driven by a U6 promoter (Figures 1 and 2).

The DNA transduced by the integrating lentivirus integrates randomly into the cellular genome to express both 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.

Applications

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

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

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

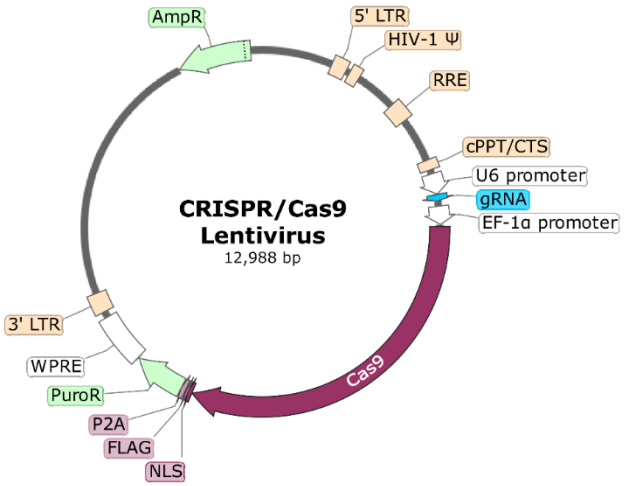


Figure 1. Schematic of the Lenti-vector used to generate the PD-1 CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
PD-1	PD-1-1	CGTGTCACACAACTGCCCAA
PD-1	PD-1-2	GCCCACGACACCAACCACCA
PD-1	PD-1-3	CCCTTCGGTCACCACGAGCA
PD-1	PD-1-4	CACCTACCTAAGAACCATCC

Figure 2: List of sgRNA Sequences in the PD-1 CRISPR/Cas9 Lentivirus.

Storage Conditions

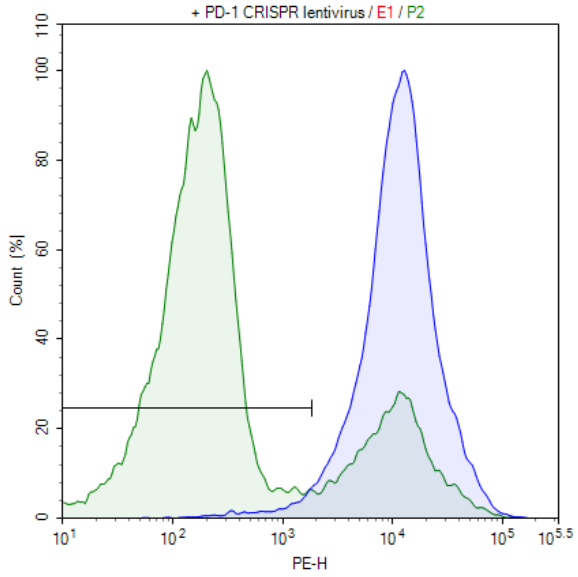
Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the lentiviruses at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with the SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Validation Results

A.

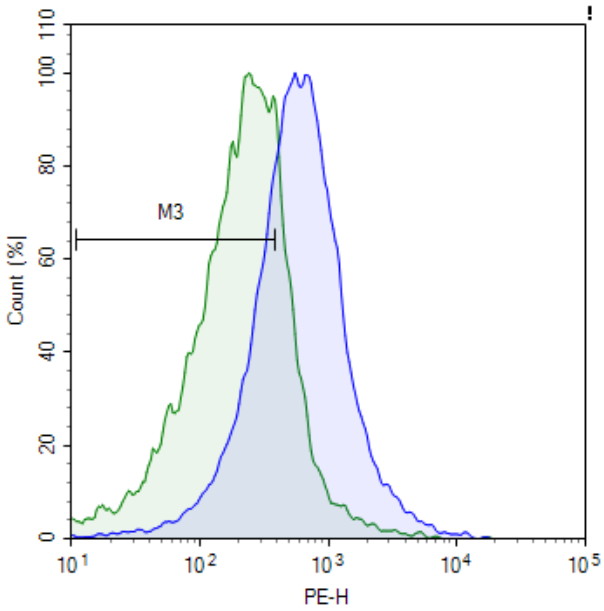


B.

Sample	Gate #	% of Cell Population
PD-1 NFAT-Reporter Jurkat	M4	2.87%
PD-1 NFAT-Reporter Jurkat cells transduced with PD-1 CRISPR/Cas9 Lentivirus	M4	75.51%

Figure 3. Knock-down of PD-1 in PD-1 Over-Expressing Jurkat cells. **A.** PD-1 NFAT-Reporter Jurkat cells (BPS Bioscience #60535) were transduced via spinoculation with 5,000,000 TU/well of PD-1 CRISPR/Cas9 lentivirus, corresponding to an MOI of approximately 5-10. 72 hours after transduction, cells were stained with PE anti-human PD-1 antibody (BioLegend, #621608) and analyzed by flow cytometry. Parental PD-1 NFAT-Reporter Jurkat cells are shown in blue, and the transduced cells are shown in green. **B**. Percentages of cell populations captured in Gate M4.

A.



B.

Sample	Gate #	% of Cell Population
Primary T cells	M3	29.37%
Primary T transduced with PD-1 CRISPR/Cas9 Lentivirus	M3	70.66%

Figure 4: Knock-down of PD-1 in primary T cells using PD-1 CRISPR/Cas9 Lentivirus. **A.** Primary T cells (1 million cells per well) were transduced via spinoculation with 5,000,000 TU/well of PD-1 CRISPR/Cas9 lentivirus, corresponding to an MOI ≥50. 72 hours after transduction, cells were stained with PE-labeled anti-human PD-1 antibody (BioLegend, # 621608) and analyzed by flow cytometry. Non-transduced primary T cells are shown in blue, and the transduced cells are shown in green. **B.** Percentages of cell populations captured in Gate M3.

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.

Related Products

Products	*Catalog #*	*Size*
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 (S. pyogenes)	100206	50 µg
TCR Knockout NFAT-Luciferase Reporter Jurkat Recombinant Cell Line	79887	2 vials
PD-1 - HEK293 Recombinant Cell Line	60680	2 vials
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials