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Data Sheet

PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)

Catalog #: 78059

Product 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 cancers, 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 cancers, 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 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 non-integrating lentivirus is made with a mutated integrase, resulting in only transient expression of the Cas9 and sgRNA. Although using the non-integrating lentivirus results in lower knockdown efficiency, the Cas9 isn't permanently expressed, which lowers the risk of off-targeting, and there are no random integrations into the cell's genome. Knockout cell lines can still be generated following cell sorting or limited dilution, because even though the Cas9 and sgRNA expression is transient, the changes in the genomic DNA from the Cas9 nuclease activity and NHEJ repair are permanent.

Application

1. Transient knock-down of PD-1 in a target cell pool.
2. Generation of stable PD-1 knock-out cells using transient puromycin selection (48h maximum) followed by limited dilution.

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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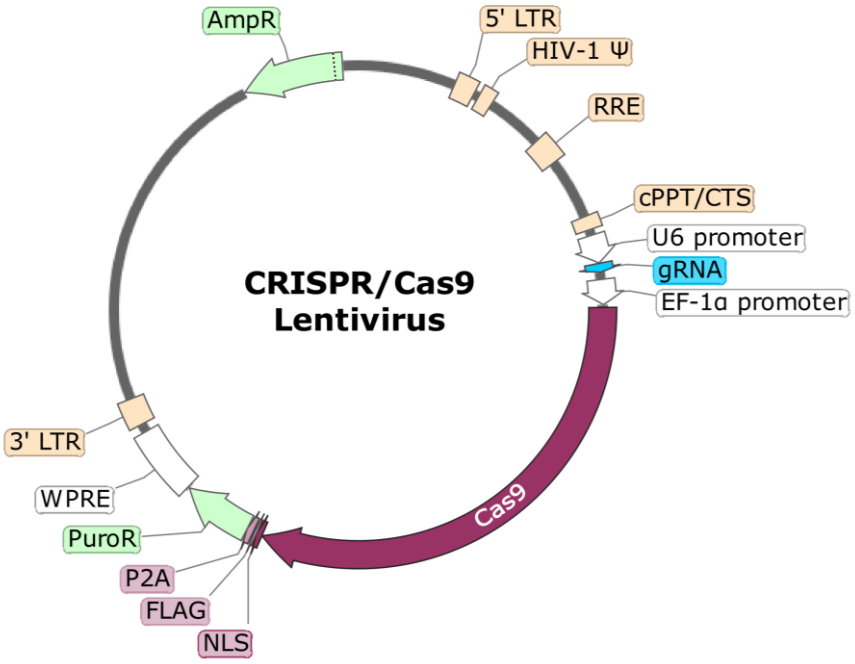

Figure 1. Schematic of the Lenti-vector used to generate the PD-1 CRISPR/Cas9 Lentivirus.

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

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

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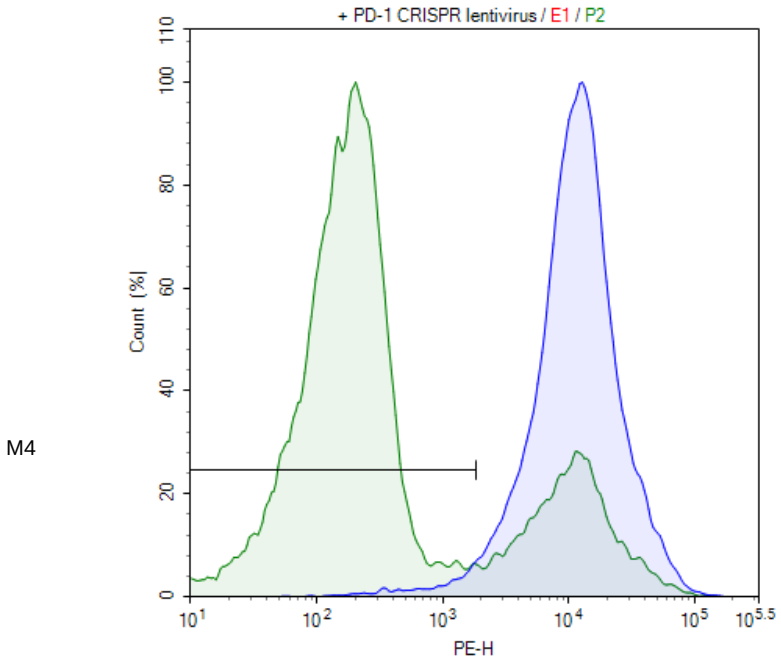
Level 2 facility. BPS recommends following all federal, state, local, and institutional regulations and using all appropriate safety precautions.

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, Cat #60535) were transduced via spinoculation with 5,000,000 TU/well of PD-1 CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE anti-human PD-1 antibody (BioLegend, #621608) and analyzed by FACS. Parental PD-1 NFAT-Reporter Jurkat cells are shown in blue, and the transduced cells are shown in green. **B.** Graph comparing the percentages of cell populations encapsulated by Gate M4.

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Related Products

Product	Cat. #	Size
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
TCR Knockout NFAT-Luciferase Reporter Jurkat 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

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.