6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

CTLA4 CRISPR/Cas9 Lentivirus (Integrating)

Catalog #: 78054

Description

CTLA4 (Cytotoxic T-Lymphocyte Associated Protein), also known as CD152, is a protein receptor that functions as an immune checkpoint. It is expressed by activated T-cells and transmits an inhibitory signal to T-cells. CTLA4 is homologous to the T-cell co-stimulatory protein CD28, and both molecules bind to CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells. CTLA4 binds CD80 and CD86 with greater affinity and avidity than CD28, thus enabling it to out-compete CD28 for its ligands and act as an "off" switch when bound to CD80 or CD86. CTLA4 is an important immunotherapy target for the treatment of cancer and autoimmune diseases.

The CTLA4 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 CTLA4, GenBank Accession #NM_005214, 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 CTLA4 in a target cell pool.
2. Generation of a stable CTLA4 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 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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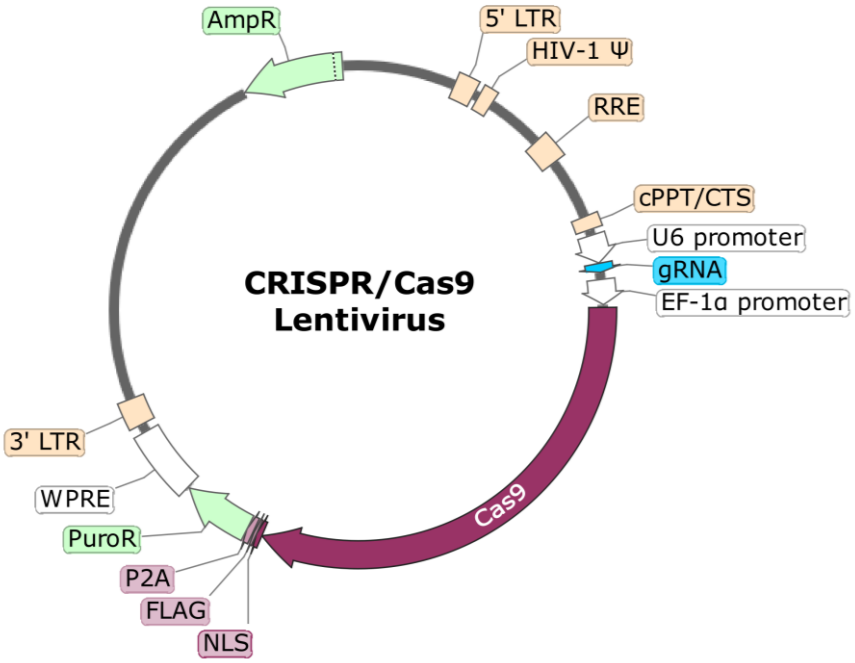

Figure 1. Schematic of the Lenti-vector used to generate the CTLA4 CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
CTLA4	CTLA4-1	TGCCCAGGTAGTATGGCGGT
CTLA4	CTLA4-2	CAAGTGAACCTCACTATCCA
CTLA4	CTLA4-3	TTCCATGCTAGCAATGCACG
CTLA4	CTLA4-4	GTGTGTGAGTATGCATCTCC

Figure 2. List of sgRNA Sequences in the CTLA4 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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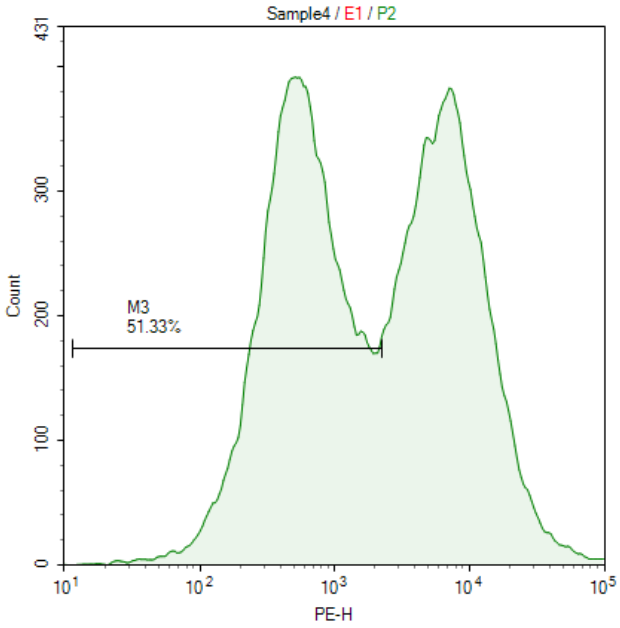

Figure 3. Knock-down of CTLA4 in CTLA4 Over-Expressing Jurkat cells.
CTLA4 IL-2-Reporter Jurkat cells (BPS Bioscience, Cat #79525) were transduced via spinoculation with 5,000,000 TU/well of CTLA4 CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE anti-human CTLA4 antibody (BioLegend, #349905) and analyzed by FACS. The cell population selected by gate M3 represents the cells in which CTLA4 expression is knocked down.

Related Products

Product	Cat. #	Size
CTLA4 CRISPR/Cas9 Lentivirus (Non-Integrating)	78061	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
CTLA4 - HEK293 Recombinant Cell Line	60681	2 vials
CTLA4 / IL-2 Reporter - Jurkat Recombinant Cell Line	79525	2 vials
Anti-CTLA4 Neutralizing Antibody	71212	100 µg
CTLA4 (CD152), Fc-fusion (IgG1)	71149-1	100 µg

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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