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Data Sheet

LAG3 CRISPR/Cas9 Lentivirus (Non-Integrating)

Catalog #: 78060

Description

Lymphocyte-activation gene 3 (LAG3, CD223) is a cell surface protein that belongs to the immunoglobulin (Ig) superfamily. LAG3 is expressed on activated T-cells, Natural Killer cells, B-cells, and plasmacytoid dendritic cells. Its main ligand is the MHC class II, to which it binds with higher affinity than CD4. It negatively regulates cellular proliferation, activation, and homeostasis of T-cells in a similar fashion as CTLA-4 and PD-1, and has been reported to play a role in T-reg suppressive function. A number of LAG3 antibodies are in preclinical development for the treatment of cancer and autoimmune disorders. LAG3 may be a better immune checkpoint inhibitor target than CTLA-4 or PD-1, because antibodies targeting CTLA-4 or PD-1 only activate effector T-cells while failing to inhibit T-reg activity, whereas an antagonist LAG3 antibody can both activate effector T-cells (by downregulating the LAG3 inhibiting signal) and inhibit induced (i.e. antigen-specific) T-reg suppressive activity.

The LAG3 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 LAG3 (GenBank Accession #NM_002286) driven by a U6 promoter (Figures 1 and 2).

Note: unlike Human LAG3 CRISPR/Cas9 Lentivirus (Integrating) (BPS Bioscience, #78053), the Human LAG3 CRISPR/Cas9 Lentivirus (Non-Integrating) is made with a mutated Integrase, resulting in only transient expression of the Cas9 and LAG3-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 LAG3 in a target cell pool.
2. Generation of stable LAG3 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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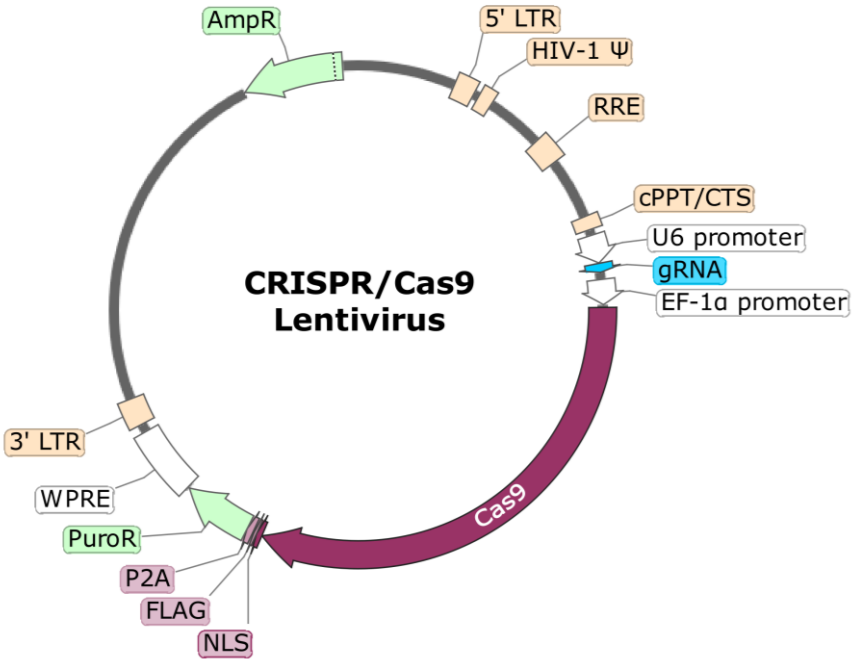

Figure 1. Schematic of the Lenti-vector used to generate the LAG3 CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
LAG3	Lag3-1	GTTCCGGAACCAATGCACAG
LAG3	Lag3-2	GGGAGTTACCCAGAACAGTG
LAG3	Lag3-3	CGTCCCGCCCCACATACTCG
LAG3	Lag3-4	GCTCACATCCTCTAGTCGAA

Figure 2. List of sgRNA Sequences in the LAG3 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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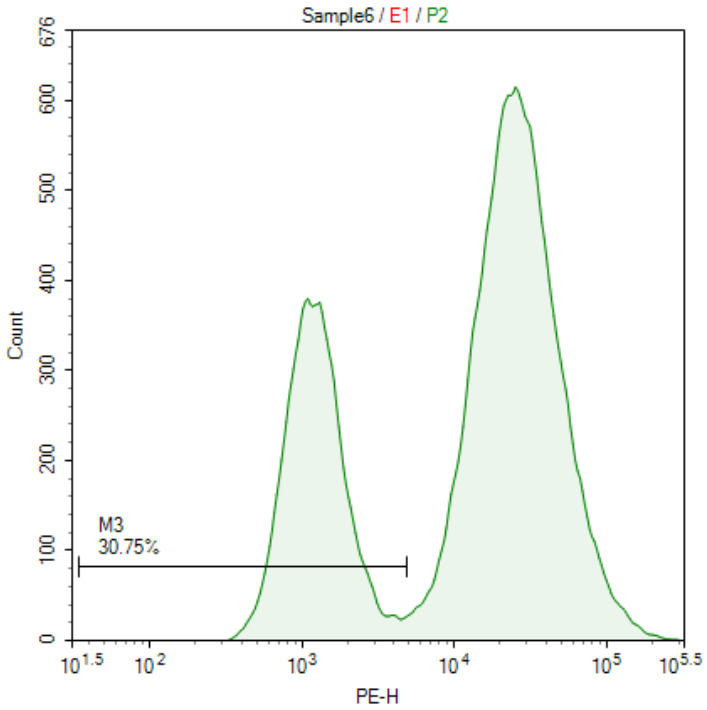

Figure 3. Knock-down of Lag3 in Lag3 Over-Expressing Jurkat cells.
LAG3 over-expressing IL-2 Reporter Jurkat cells (BPS Bioscience, #79813) were transduced via spinoculation with 5,000,000 TU/well of LAG3 CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE anti-human LAG3 antibody (BioLegend, #369305) and analyzed by FACS. M3 indicates the population of cells in which LAG3 is knocked-down. This cell population can be increased following puromycin selection.

Related Products

Product	**Cat. #**	**Size**
LAG3 CRISPR/Cas9 Lentivirus (Integrating)	78053	500 µl x 2
LAG3 / IL-2 Reporter - Jurkat Recombinant Cell Line	79813	2 vials
LAG3 / NFAT Reporter - Jurkat Recombinant Cell Line	71278	2 vials
Anti-LAG3, Neutralizing Antibody	71213	100 µg
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

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.