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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

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

LAG3 CRISPR/Cas9 Lentivirus (Integrating)

Catalog #: 78053

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).

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 LAG3 in target cells.
2. Generation of a stable LAG3 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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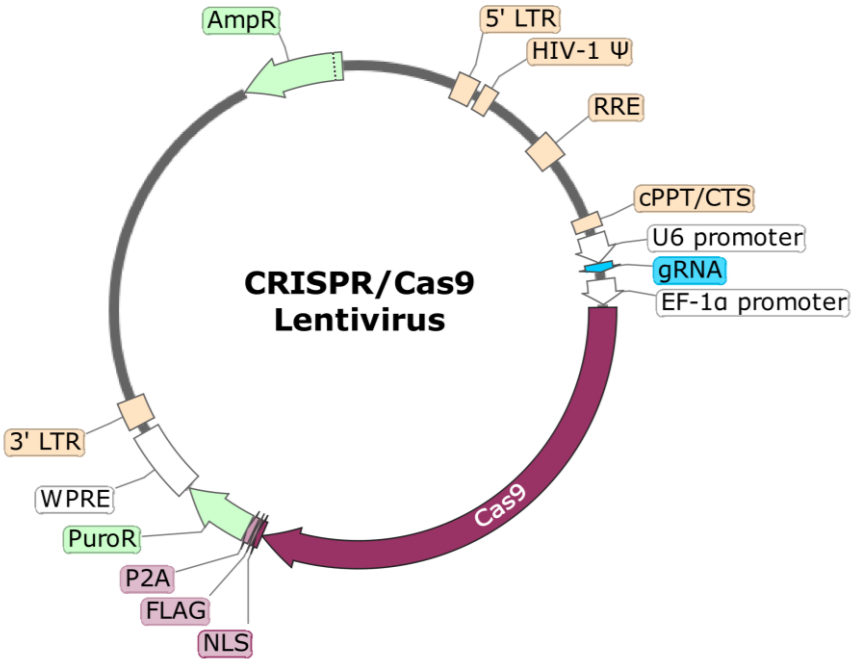

Figure 1. Schematic of the Lenti-vector used to generate the LAG3 CRISPR/Cas9 Lentivirus.

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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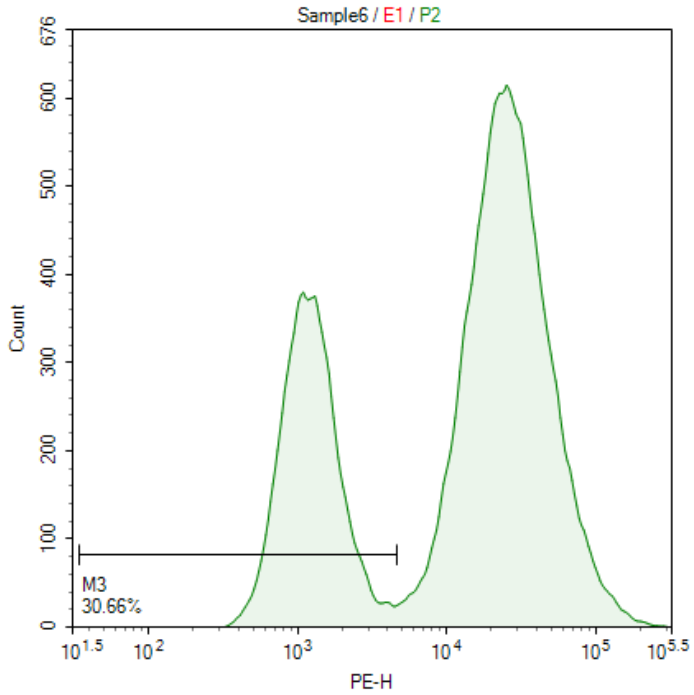

Figure 3. Knock-down of LAG3 in LAG3 Over-Expressing Jurkat cells.
LAG3 over-expressing IL-2 Reporter Jurkat cells (BPS Bioscience, #79813) were transduced via spinoculation with 5,000,000 TU/well of LAG3 CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE anti-human LAG3 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 (Non-Integrating)	78060	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 (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)	100206-1	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.