Description

LAG3 Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses result in the expression of LAG3 (lymphocyte-activation gene 3) (NM_002286.6), also known as CD223, driven by an EF1a promoter. The lentiviruses also contain a Blasticidin selection marker (Figure 1).

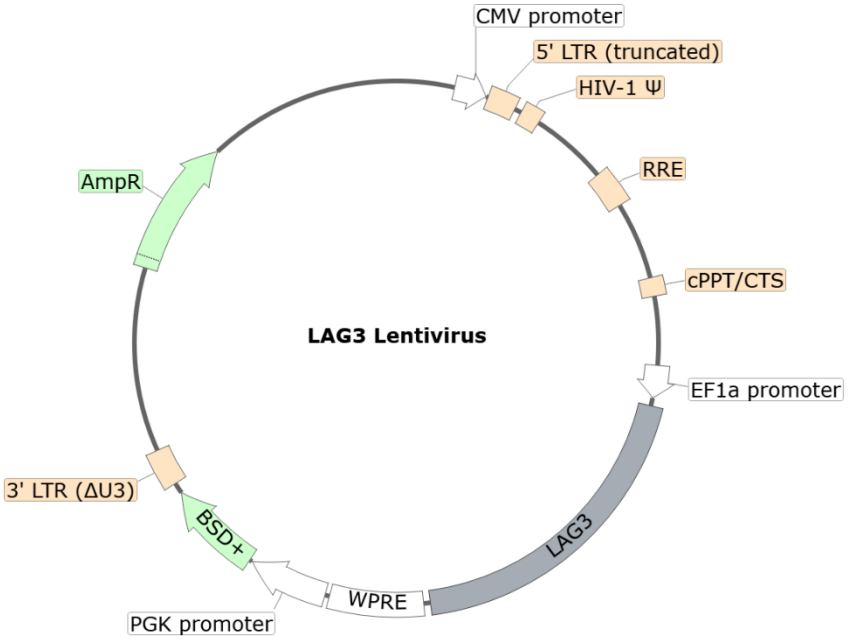

Figure 1. Schematic of the lenti-vector used to generate LAG3 Lentivirus.

Background

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 MHC (major histocompatibility complex) 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 to CTLA-4 (cytotoxic T-lymphocyte associated protein 4) and PD-1 (programmed cell death protein 1) and has been reported to play a role in the Treg suppressive function. Several 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 since antibodies against these two checkpoints only activate effector T cells but don't inhibit Treg activity. Antagonist LAG3 antibodies can both activate effector T cells (by downregulating the LAG3 inhibiting signal) and inhibit induced (i.e. antigen-specific) Treg suppressive activity.

Application(s)

- Expression of human LAG3 in cells of interest.
- Generate human LAG3 expressing cell pools or stable cell lines by Blasticidin selection.

Formulation

The lentivirus particles were produced in HEK293T cells. They are supplied in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a 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.

Notes

To generate a LAG3 stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of Blasticidin (as pre-determined from a killing curve, https://bpsbioscience.com/kill-curve-protocol), for antibiotic selection of transduced cells, followed by clonal selection.

Validation Data

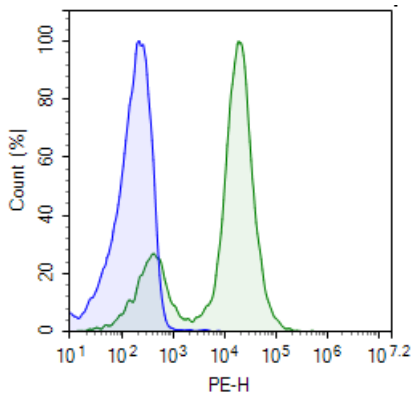

Figure 2. Expression of LAG3 in Jurkat cells transduced with LAG3 Lentivirus.
Approximately 100,000 Jurkat cells were transduced with 1 x 10^6 TU (100 µl of 10^7 TU/ml) of LAG3 Lentivirus in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (#78939). 48 hours post-transduction, the cells were selected with 10 µg/ml of blasticidin, and the blasticidin resistant cell pool was stained with Anti-LAG3 Antibody, PE-Labeled (#71226) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity. Blue, Jurkat cells; Green, Jurkat cells transduced with LAG3 Lentivirus.

Data is representative.

Sequence
Human LAG3 sequence **(**NM_002286.6)

MWEAQFLGLLFLQPLWVAPVKPLQPGAEVPVVWAQEGAPAQLPCSPTIPLQDLSLLRRAGVTWQHQPDSGPPAAAP
GHPLAPGPHPAAPSSWGPRPRRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHL
RDRALSCRLRLRLGQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQGRVPVRESPHHHLAESFLFLPQV
SPMDSGPWGCILTYRDGFNVSIMYNLTVLGLEPPTPLTVYAGAGSRVGLPCRLPAGVGTRSFLTAKWTPPGGGPDLLV
TGDNGDFTLRLEDVSQAQAGTYTCHIHLQEQQLNATVTLAIITVTPKSFGSPGSLGKLLCEVTPVSGQERFVWSSLDTPS
QRSFSGPWLEAQEAQLLSQPWQCQLYQGERLLGAAVYFTELSSPGAQRSGRAPGALPAGHLLLFLILGVLSLLLLVTGAF
GFHLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQEPEPEPEPEPEPEPEPEPEQL

References
Aggarwal V., *et al.*, 2023 *Nature Immunology* 24:1415-1422.

Troubleshooting Guide
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
LILRB2 Lentivirus	82451	500 µl x 2
LILRB4 Lentivirus	82452	500 µl x 2
Anti-LAG3 Neutralizing Antibody	71219	100 µg
LAG3 CRISPR/ Cas9 Lentivirus (Integrating)	78053	500 µl x 2
LAG3 CRISPR/ Cas9 Lentivirus (Non-Integrating)	78060	500 µl x 2
LAG3/ NFAT Reporter – Jurkat Recombinant Cell Line	71278	2 vials

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