

Data Sheet

TIGIT CRISPR/Cas9 Lentivirus (Integrating)

Catalog #: 78058

Description

TIGIT (T-cell immunoreceptor with Ig and ITIM domains; VSTM3; VSIG9) is a co-inhibitory receptor that is highly expressed in Natural Killer (NK) cells and activated CD4+, CD8+, and regulatory T-cells. Interaction with the Poliovirus Receptor (PVR; CD155) on antigen presenting cells, such as dendritic cells, recruits either the Src homology (SH) domain-containing tyrosine phosphatases SHP1 and SHP2, or the Inositol phosphatase SHIP1 and SHIP2, to the TIGIT ITIM domain. This increases IL-10 release and suppresses NF- κ B and NFAT T-cell receptor (TCR) signaling, which blocks T-cell proliferation and cytokine production. TIGIT also serves as a competitive inhibitor of CD226, a costimulatory receptor for CD155. TIGIT-targeting antibodies which block this T-cell intrinsic inhibitory effect have shown enhanced anti-tumor and anti-viral functions in preclinical studies.

The TIGIT 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 TIGIT (GenBank Accession #NM_173799) 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 TIGIT in a target cell pool.
2. Generation of a stable TIGIT 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 μ l x 2) of lentivirus at a titer $\geq 1 \times 10^6$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

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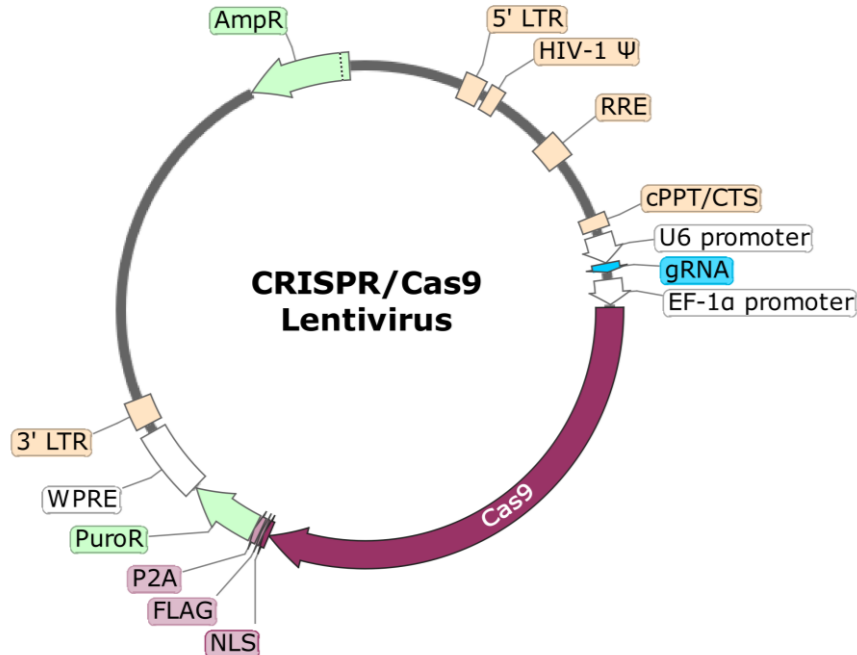


Figure 1. Schematic of the Lenti-vector used to generate the TIGIT CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
TIGIT	TIGIT-1	CATCTGCACAGCAGTCATCG
TIGIT	TIGIT-2	CAGGCACAATAGAAACAACG
TIGIT	TIGIT-3	GCTGACCGTGAACGATACAG
TIGIT	TIGIT-4	ACCCTGATGGGACGTACT

Figure 2. List of sgRNA Sequences in the TIGIT 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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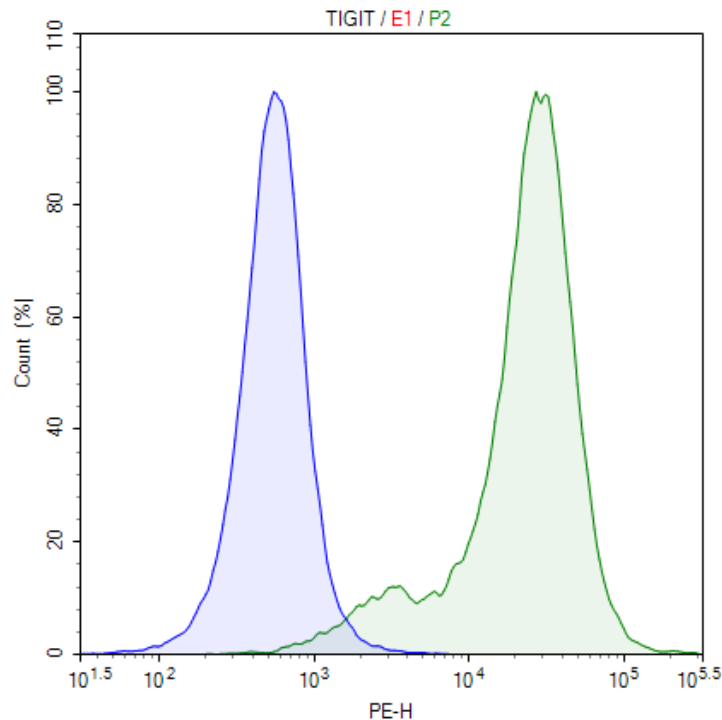


Figure 3. Knock-down of TIGIT in TIGIT Over-Expressing HEK2993 cells.

TIGIT over-expressing HEK2993 cells (BPS Bioscience, #79332) were transduced via spinoculation with 5,000,000 TU/well of TIGIT CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE-labeled anti-human TIGIT antibody (BioLegend, #372703) and analyzed by FACS. Parental TIGIT over-expressing HEK2993 cells are shown in green, and the transduced cells are shown in blue.

Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)	78065	500 µl x 2
TIGIT / NFAT Reporter - Jurkat Cell Line	60538	2 vials
TIGIT - HEK2993 Recombinant Cell Line	79332	2 vials
Anti-TIGIT Neutralizing Antibody	71340	100 µg
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2
Cas9, His-tag (<i>S. pyogenes</i>)	100206-1	50 µg
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	79887	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.

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