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Data Sheet

TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)

Catalog #: 78065

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-κB 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).

Note: unlike human TIGIT CRISPR/Cas9 Lentivirus (Integrating) (BPS Bioscience, #78058), the human TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating) is made with a mutated Integrase, resulting in only transient expression of the Cas9 and TIGIT 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 TIGIT in a target cell pool.
2. Generation of stable TIGIT 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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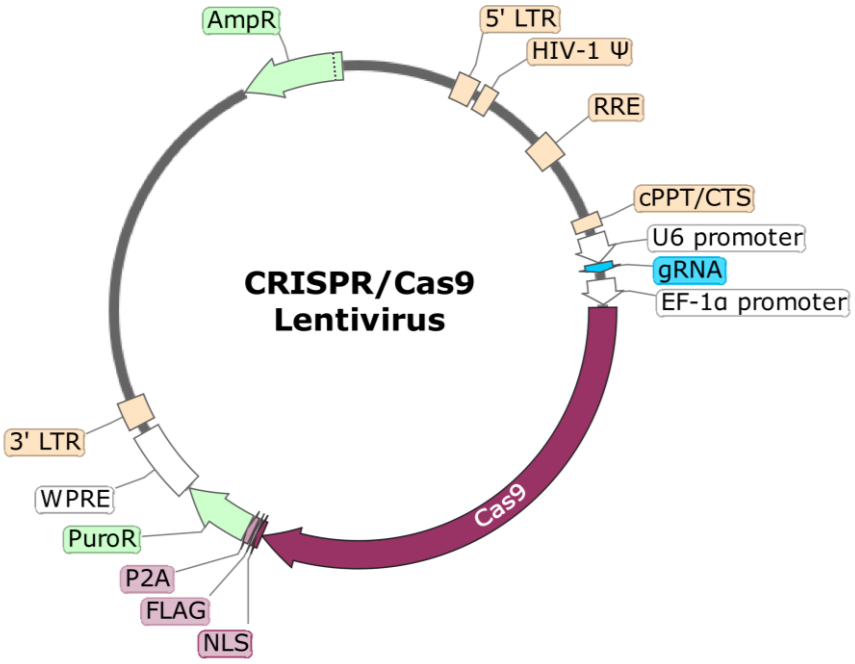

Figure 1. Schematic of the Lenti-vector used to generate the TIGIT CRISPR/Cas9 Lentivirus.

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

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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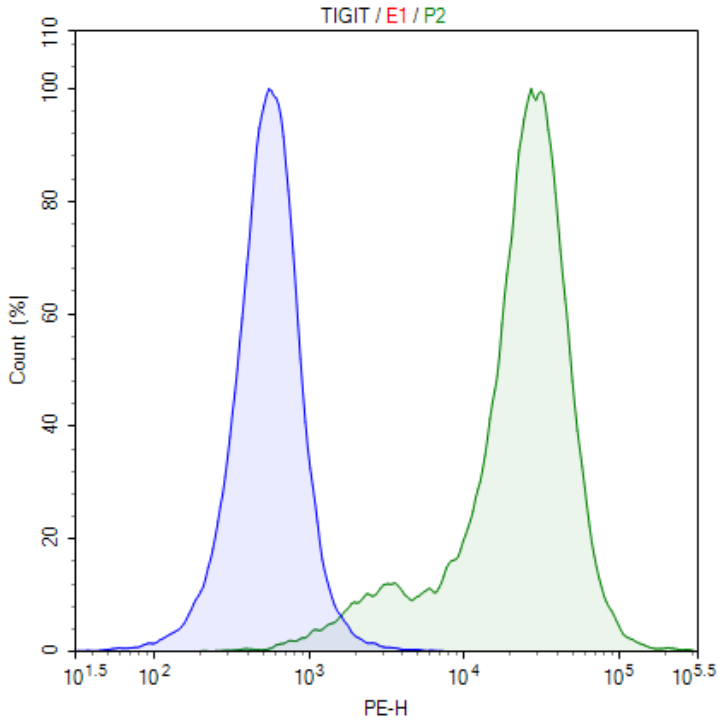

Figure 3. Knock-down of TIGIT in TIGIT Over-Expressing HEK293 cells.
TIGIT over-expressing HEK293 cells (BPS Bioscience, #79332) were transduced via spinoculation with 5,000,000 TU/well of TIGIT CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE-labeled anti-human TIGIT antibody (BioLegend, #372703) and analyzed by FACS. Parental TIGIT over-expressing HEK293 cells are shown in green, and the transduced cells are shown in blue.

Related Products

Product	**Cat. #**	**Size**
TIGIT CRISPR/Cas9 Lentivirus (Integrating)	78058	500 µl x 2
TIGIT / NFAT Reporter - Jurkat Cell Line	60538	2 vials
TIGIT - HEK293 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 (S. pyogenes)	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.