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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

TCR CRISPR/Cas9 Lentivirus (Non-Integrating)

Catalog #: 78062

Product Description

The T-Cell Receptor (TCR) is found on the surface of T-cells and is responsible for recognizing antigens bound to MHC (Major Histocompatibility Complex) molecules. Activation of the TCR results in activation of downstream NFAT signaling. The TCR consists of a heterodimer of two different protein chains, of which the alpha (α) and beta (β) chains are the predominant chains.

The TCR 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 TRAC (T-Cell Receptor Alpha Constant) and human TRBC1 (T-Cell Receptor Beta Constant 1) regions of the α and β chains.

The non-integrating lentivirus is made with a mutated integrase, resulting in only transient expression of the Cas9 and sgRNA. Although using the non-integrating lentivirus results in lower knockdown efficiency, the Cas9 isn't permanently expressed, which lowers the risk of off-targeting, and there are no random integrations into the cell's genome. Knockout cell lines can still be generated following cell sorting or limited dilution, because even though the Cas9 and sgRNA expression is transient, the changes in the genomic DNA from the Cas9 nuclease activity and NHEJ repair are permanent.

Application

1. Transient knock-down of TCR in a target cells pool.
2. Generation of a stable TCR 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 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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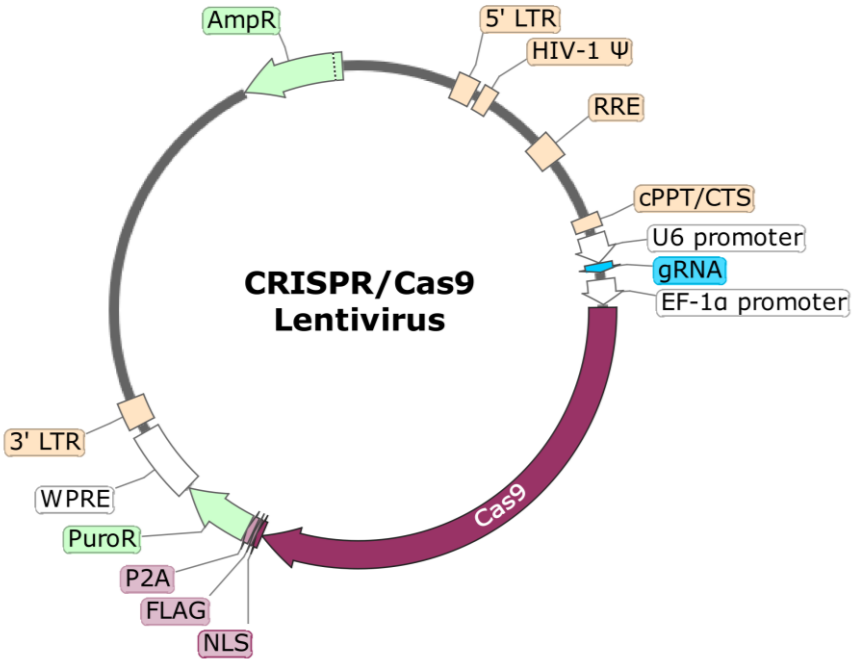

Figure 1. Schematic of the Lenti-vector used to generate the TCR CRISPR/Cas9 Lentivirus.

Gene Target:	Primer ID:	sgRNA Sequence:
TRAC	TCR-1	AGAGTCTCTCAGCTGGTACA
TRAC	TCR-2	TGTGCTAGACATGAGGTCTA
TRBC1	TCR-3	GGAGAATGACGAGTGGACCC
TRBC1	TCR-4	GCAGTATCTGGAGTCATTGA

Figure 2. List of sgRNA Sequences in the TCR 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.

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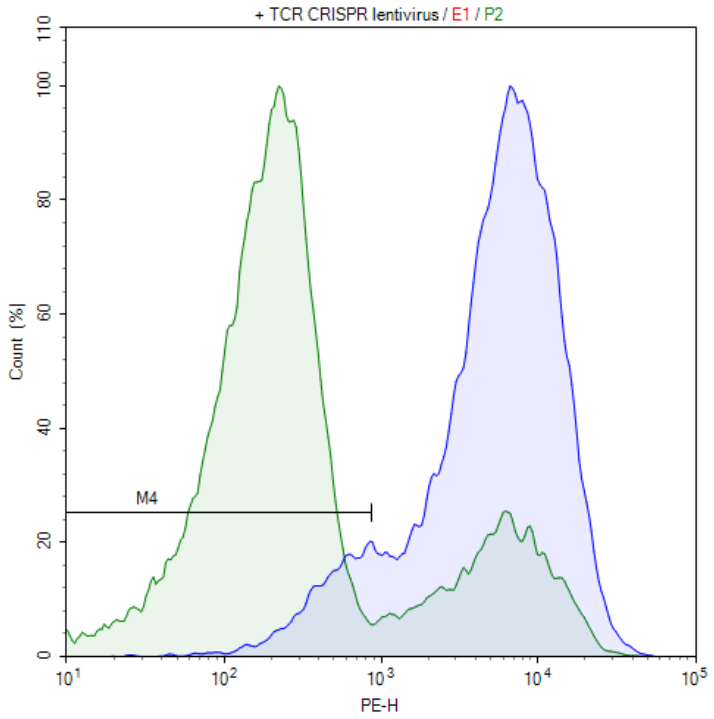
Biosafety
None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells. Although the pseudotyped lentiviruses are replication-incompetent, they do require the use of a Biosafety Level 2 facility. BPS recommends following all federal, state, local, and institutional regulations and using all appropriate safety precautions.

A.



B.

Sample	Gate #	% of Cell Population
Jurkat Parental cells	M4	9.79%
Jurkat cells transduced with TCR CRISPR/Cas9 Lentivirus	M4	75.30%

Figure 3. Knock-down of TCR in Jurkat cells.
A. Jurkat cells were transduced via spinoculation with 5,000,000 TU/well of TCR CRISPR/Cas9 lentivirus. 72 hours after transduction, cells were stained with PE anti-human TCR antibody (BioLegend, #306708) and analyzed by FACS. Parental Jurkat cells are shown in blue, and the transduced cells are shown in green. **B.** Graph comparing the percentages of cell populations encapsulated by Gate M4.

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Related Products

Product	Cat. #	Size
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Integrating)	78052	500 µl x 2
PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78059	500 µl x 2
Cas9, His-tag (S. pyogenes)	100206-1	50 µg
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	79887	2 vials
TCR Activator - Raji Cell Line	60556	2 vials
TCR Activator - CHO Cell Line	60539	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.