

Description

CD5 is a member of the scavenger receptor cysteine-rich (SRCR) superfamily and is associated with the immune system. This protein is a transmembrane glycoprotein found on the surface of thymocytes and may act as a receptor to regulate T-cell proliferation.

The CD5 CRISPR Lentiviruses are replication incompetent, HIV-based VSV-G pseudotyped 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 5 sgRNA (single guide RNA) targeting human CD5 driven by a U6 promoter (Figures 1 and 2).

Unlike CD5 CRISPR/Cas9 Lentivirus (Integrating) (BPS Bioscience, #78119), the CD5 (Human) CRISPR/Cas9 Lentivirus (Non-Integrating) is made with a mutated Integrase, therefore the viral DNA cannot integrate into the genome, resulting in only transient expression of the Cas9 and CD5-targeting sgRNA. It is expected that this will minimize potential off-target effects caused by either prolonged expression or random integration of Cas9 and the sgRNA. A short round of puromycin selection right after transduction may increase knockout efficiency, however puromycin should not be used for more than 48 hours post-transduction due to the transient nature of expression using the non-integrating lentivirus. Efficiencies may vary, depending on the cell type and the gene of interest.

Application

- Transient knock-down of CD5 in target cells.
- Generation of stable CD5 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 μ l x 2) of firefly luciferase 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.

Storage



Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus 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, as they are expressed from packaging plasmids lacking the packing signal. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Figures and Validation Data

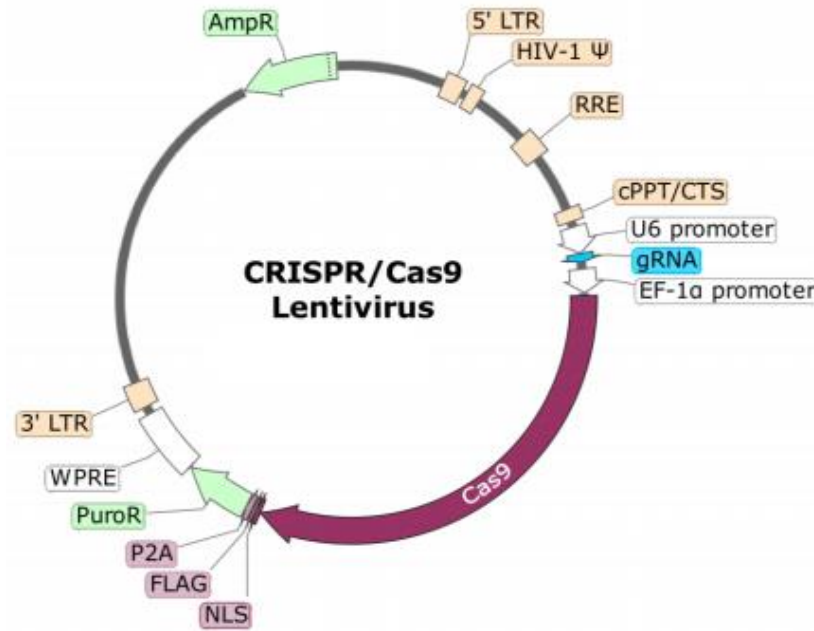


Figure 1. Schematic of the Lenti-vector used to generate the CD5 CRISPR/Cas9 Lentivirus

Gene Target:	Primer ID:	sgRNA Sequence
CD5	CD5-1	CAGCATCTGTGAAGGCACCG
CD5	CD5-2	TTTCCTGAAGCAATGCTCCA
CD5	CD5-3	AAGCGTCAAAAGTCTGCCAG
CD5	CD5-4	GCTGTAGAACTCCACCACGC
CD5	CD5-5	CGTTCCAACCTCGAAGTGCCA

Figure 2. List of sgRNA Sequences in the CD5 CRISPR/Cas9 Lentivirus

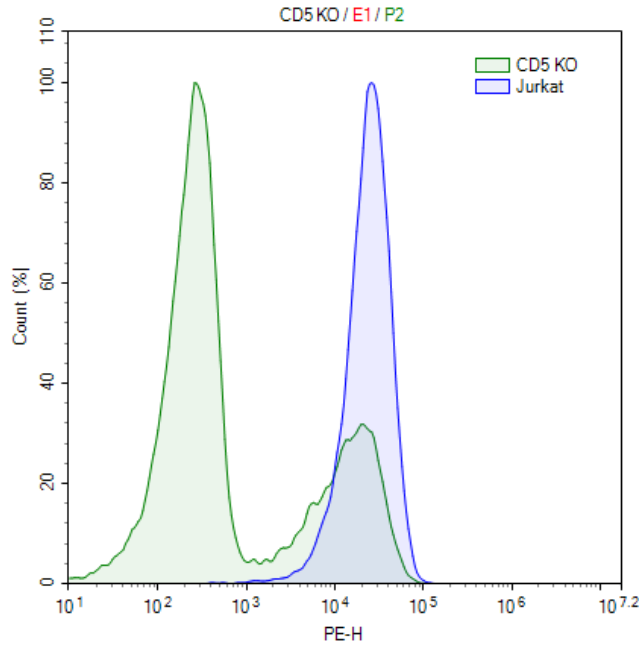


Figure 3. Knock-down of CD5 in Jurkat cells.

Jurkat cells were transduced via spinoculation with CD5 CRISPR/Cas9 lentivirus. 72 hours after transduction, without selection, cells were stained with PE-labeled anti-human CD5 antibody (BioLegend, #364013) and analyzed by flow cytometry. Parental Jurkat cells are shown in blue, and the transduced cells are shown in green.

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.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CD5 CRISPR/Cas9 Lentivirus (Integrating)	78119	500 µl x 2
CD5, Fc-Fusion, Avi-Tag, HiP™	101005-1	100 µg
CD5, Fc Fusion, Avi-Tag, Biotin-labeled, HiP™	101006-1	20 µg
CD5L, Avi-His-Tag	101007-1	100 µg