

Description

Beta-2 Microglobulin (B2M) is a required component of Major Histocompatibility Complex (MHC) class 1 molecules, which present peptide fragments from within the cell to cytotoxic T-cells as part of the adaptive immune system.

The B2M CRISPR/Cas9 Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to infect 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 RNAs) targeting human B2M driven by a U6 promoter (Figures 1 and 2).

Note: unlike B2M CRISPR/Cas9 Lentivirus (Integrating) (BPS Bioscience, #78340), the B2M CRISPR/Cas9 Lentivirus (Non-Integrating) is made with a mutated Integrase, resulting in only transient expression of the Cas9 and B2M-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.

Application

1. Transient knock-down of B2M in target cells
2. Generation of stable B2M 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 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 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 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

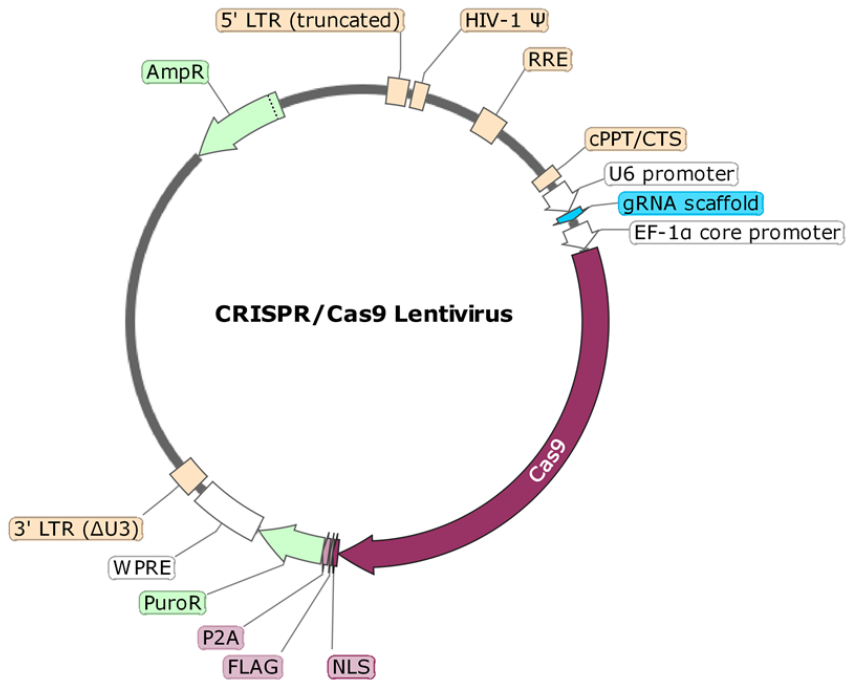


Figure 1. Schematic of the Lenti-vector used to generate the B2M CRISPR/Cas9 Lentivirus

Gene Target:	sgRNA Sequence
B2M	AAGTCAACTTCAATGTCGGA
B2M	CTGAATCTTTGGAGTACCTG
B2M	GAGTAGCGCGAGCACAGCTA
B2M	TCCTGAATTGCTATGTGTCT
B2M	GAAGTTGACTTACTGAAGAA

Figure 2. List of sgRNA Sequences in the B2M CRISPR/Cas9 Lentivirus

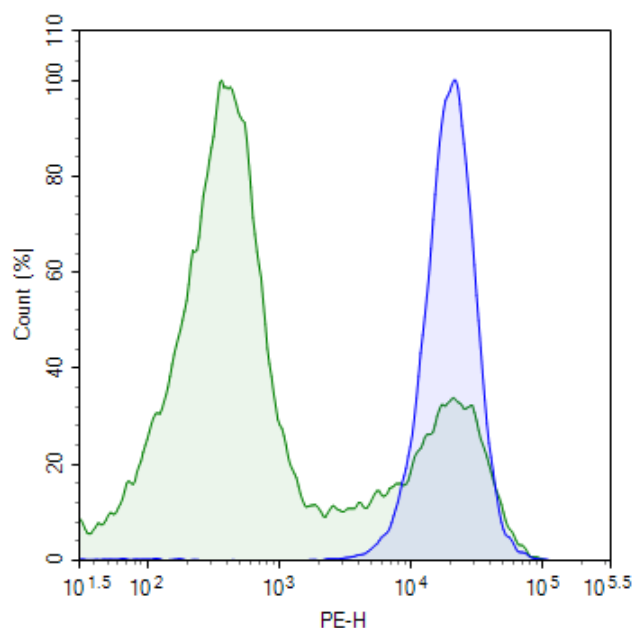


Figure 3. Knock-down of B2M in Jurkat cells.

Parental Jurkat cells were transduced via spinoculation with B2M CRISPR/Cas9 lentivirus. 24 hours after transduction, cells were selected for another 24 hours with puromycin, then stained with PE-labeled anti-human B2M antibody (BioLegend, #395703) 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>
B2M (Human) CRISPR/Cas9 Lentivirus (Integrating)	78340	500 µl x 2
FcRn (FCGRT/B2M), His-Tag (Mouse) HiP™	11349	100 µg
FcRn (FCGRT/B2M), His-Tag	71285	100 µg/1 mg
FcRn (FCGRT/B2M), His-Tag, Biotin-Labeled, (Human) HiP™	71283	25 µg/50 µg
FcRn (FCGRT/B2M), His-Avi-Tag, Biotin-Labeled (Mouse)	71286	50 µg