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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

CBL-B is an E3 ubiquitin-protein ligase which has been identified as a negative regulator of T-cell activation. Using CRISPR/Cas9 to inactivate CBL-B has been shown to be sufficient to inhibit T-cell expansion.

The CBL-B 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 CBL-B driven by a U6 promoter (Figures 1 and 2).

Note: unlike CBL-B CRISPR/Cas9 Lentivirus (Integrating) (BPS Bioscience, #78343), the CBL-B CRISPR/Cas9 Lentivirus (Non-Integrating) is made with a mutated Integrase, resulting in only transient expression of the Cas9 and CBL-B-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 CBL-B in target cells
2. Generation of stable CBL-B 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 $\geq 1 \times 10^6$ 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. Titters 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

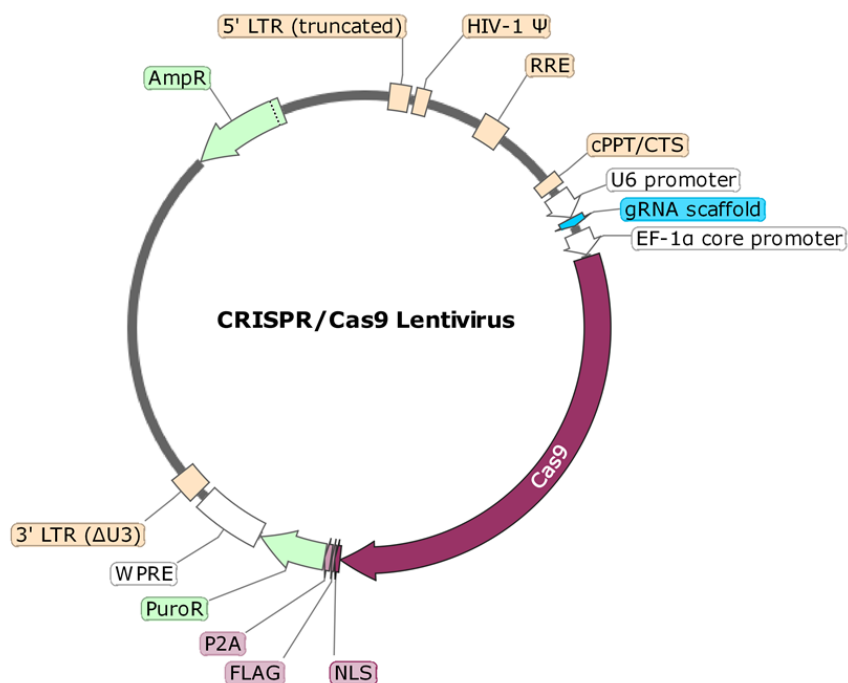


Figure 1. Schematic of the Lenti-vector used to generate the CBL-B CRISPR/Cas9 Lentivirus

| Gene Target: | sgRNA Sequence |
|--------------|------------------------|
| CBL-B | TGTGGGATGTCTCGACTCCTAG |
| CBL-B | CTTCATCTCTTGATCAAAG |
| CBL-B | TTCCGCAAAATAGAGCCCCA |
| CBL-B | TGAATTAGATCCAGGCGAGG |
| CBL-B | TGCACAGAACTATCGTACCA |

Figure 2. List of sgRNA Sequences in the CBL-B CRISPR/Cas9 Lentivirus

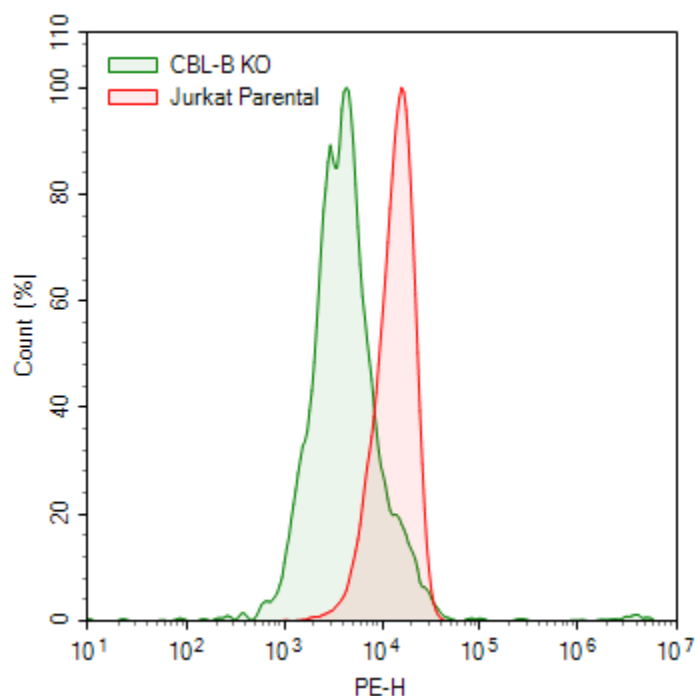


Figure 3. Knock-down of CBL-B in Jurkat cells.

Parental Jurkat cells were transduced via spinoculation with CBL-B CRISPR/Cas9 lentivirus. 24 hours after transduction, cells were selected for 24 hours with puromycin, stained with anti-human CBL-B antibody (Proteintech, #12781-1-AP) and PE-conjugated anti-Rabbit secondary antibody (BioLegend, #406421), then analyzed by flow cytometry. Parental Jurkat cells are shown in red, 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> |
|--|------------------|-------------|
| CBL-B TR-FRET Assay Kit | 79575 | 384 rxns. |
| CBL TR-FRET Assay Kit | 79786 | 384 rxns. |
| CBL-B, His-Avi-Tag | 80414 | 100 µg |
| CBL-B, GST-Tag (Human) | 80415 | 100 µg |
| CBL-c, FLAG-Tag | 100332 | 100 µg |
| CBL-B, His-Avi-Tag, Biotin-labeled (Human) | 80412-1 | 25 µg |
| CBL-B (Y363F), His-tag, Biotin-labeled (Human) | 80413-1 | 25 µg |
| CBL-B (Human) CRISPR/Cas9 Lentivirus (Integrating) | 78343 | 500 µl x 2 |