

### 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  $\mu$ 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  $-80^{\circ}\text{C}$ . 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](https://bpsbioscience.com/license) for the label license and other key information about this product.

### Troubleshooting Guide

Visit [bpsbioscience.com/lentivirus-faq](https://bpsbioscience.com/lentivirus-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

Figures and Validation Data

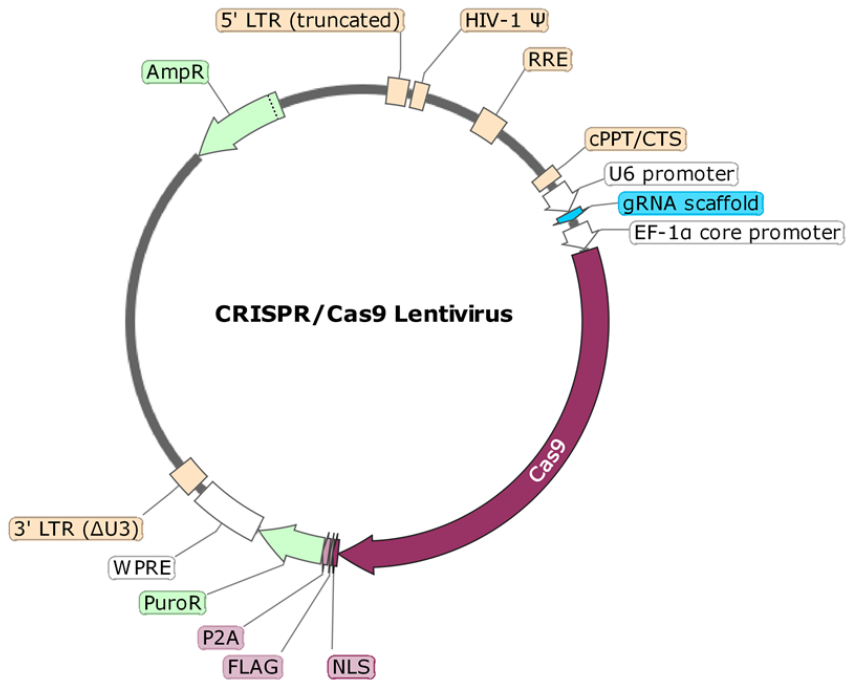


Figure 1. Schematic of the Lenti-vector used to generate the CBL-B CRISPR/Cas9 Lentivirus

Gene Target:	sgRNA Sequence
CBL-B	TGTGGGATGTCGACTCCTAG
CBL-B	CTTCATCTCTTGATCAAAG
CBL-B	TTCCGCAAATAGAGCCCA
CBL-B	TGAATTAGATCCAGGCGAGG
CBL-B	TGCACAGAACTATCGTACCA

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

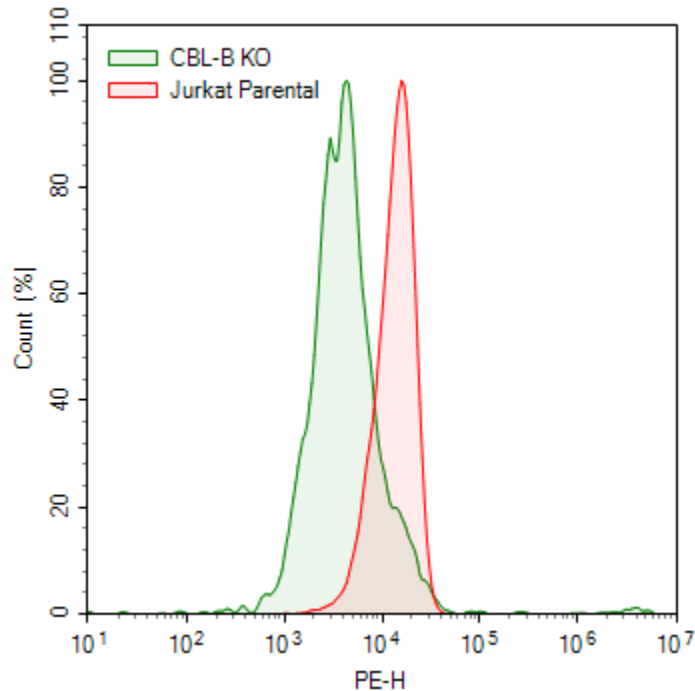


Figure 3. Knock-down of CBL-B in Jurkat cells.

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