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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Cereblon (CRBN) forms an E3 ubiquitin ligase complex which is responsible for ubiquitinating proteins that regulate various developmental processes. CRBN also binds to Calcium Activated Potassium Channel subunit alpha-1 (KCNMA1) to regulate ion transport. Moreover, mutations in CRBN may play an underlying role in tumor cells acquiring resistance to immunotherapy.

The CRBN CRISPR/Cas9 Lentiviruses are replication incompetent, HIV-based VSV-G pseudo-typed lentiviral particles that are ready to transduce 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 CRBN.

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.

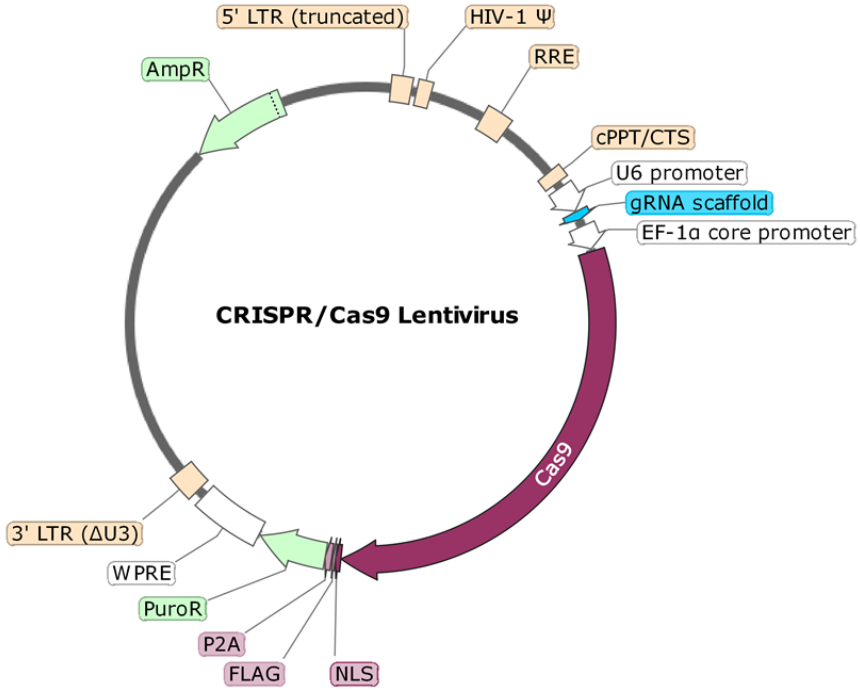

Figure 1: Schematic of the lenti-vector used to generate the CRBN CRISPR/Cas9 Lentivirus.

Gene Target:	sgRNA Sequence:
CRBN	ACCAATGTTCATATAAATGG
CRBN	CTGACTGTGTTCTTAGCTCA
CRBN	TTACATACTGTATGTGATGT
CRBN	TTCTAATTGAACTGCAGACA
CRBN	TCAAGAAACAGCTACGTGAA

Table 1: List of sgRNA Sequences in the CRBN CRISPR/Cas9 Lentivirus.

Application(s)

- Transient knockdown of CRBN in target cell pools
- Generation of stable CRBN knockout cells following transient puromycin selection (48h maximum) followed by limiting dilution cloning.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥1 x 10^7 TU/ml. The titer will vary with each lot; the exact value will be 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

The lentiviruses are produced with the SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction. 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 and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Figures and Validation Data

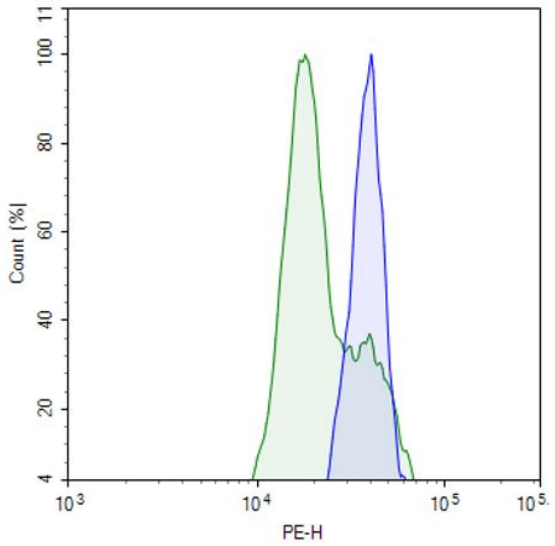

Figure 2: Knockdown of CRBN in Jurkat cells using CRBN CRISPR/Cas9 Lentivirus.
Jurkat cells were transduced via spinoculation with 8 x 10^7 TU/well of CRBN CRISPR/Cas9 lentivirus, corresponding to an MOI of approximately 5-10. 48 hours after transduction, cells were stained with PE-labeled anti-human CRBN antibody (ThermoFisher #PA5-98707) and analyzed by flow cytometry. Non-transduced, parental Jurkat cells are shown in blue, and the transduced cells are shown in green.

Troubleshooting Guide

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

License Disclosure

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

Products	Catalog #	Size
CRBN CRISPR/Cas9 Lentivirus (Integrating)	78517	500 µl x 2
Cas9 Lentivirus (Puromycin Selection)	78066	500 µl x 2
Cas9 Lentivirus (Neomycin Selection)	78432	500 µl x 2
Cereblon/DDB1/Cul4A/Rbx1 Complex Recombinant	100329	50 µg