

Description

FCGR2A1 (Low Affinity Ila Receptor (FcγRIIa), also known as CD32a) encodes one member of a family of immunoglobulin Fc receptor genes found on the surface of many immune response cells such as macrophages and neutrophils. It is involved in the process of phagocytosis and clearing immune complexes. FCGR2A is involved in activating cellular responses, while FCGR2B is inhibitory, and the balance between these receptors regulates immune activation and tolerance.

The FCGR2A CRISPR 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 RNA) targeting human FCGR2A driven by a U6 promoter (Figures 1 and 2).

Unlike FCGR2A CRISPR/Cas9 Lentivirus (Integrating) (BPS Bioscience, #78207), the FCGR2A (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 Cas9 and CD32a-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. Note: Efficiencies may vary, depending on the cell type and the gene of interest.

Application

1. Transient knock-down of FCGR2A in target cells
2. Generation of a stable FCGR2A knock-out cell pool following puromycin selection
3. Generation of a stable FCGR2A knock-out cell line following 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. 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

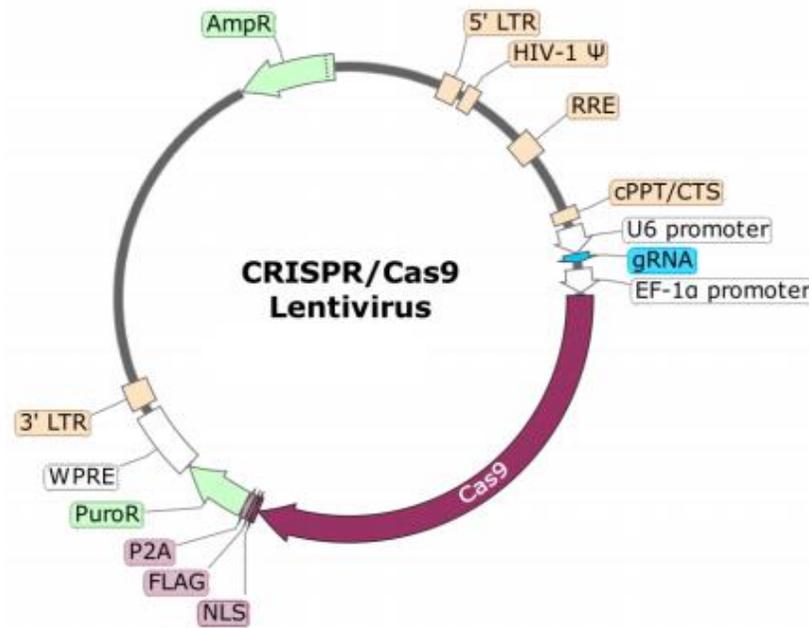


Figure 1. Schematic of the Lenti-vector used to generate the FCGR2A CRISPR/Cas9 Lentivirus

Gene Target:	Primer ID:	sgRNA Sequence
FCGR2A1	CD32a-1	TGGAGCACGTTGATCCACGG
FCGR2A1	CD32a-2	AGGGAGAAACCATCATGCTG
FCGR2A1	CD32a-3	GCTTGTGGGATGGAGAAGGT
FCGR2A1	CD32a-4	AGCAGCAGCAAACACTGTCAA
FCGR2A1	CD32a-5	AAAGCACAGTCAGATGCACA

Figure 2. List of sgRNA Sequences in the FCGR2A CRISPR/Cas9 Lentivirus

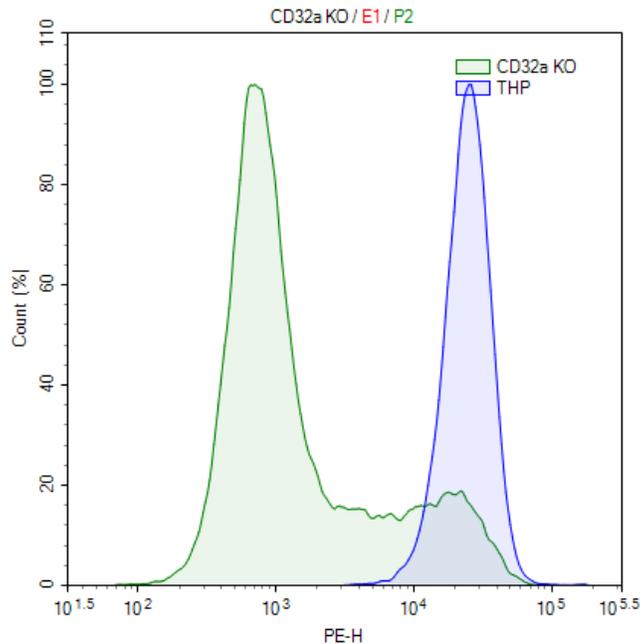


Figure 3. Knock-down of FCGR2A in THP-1 cells.

Parental THP-1 cells were transduced via spinoculation with FCGR2A CRISPR/Cas9 lentivirus. 72 hours after transduction, without selection, cells were stained with PE-labeled anti-human FCGR2A antibody (BioLegend, #303205) and analyzed by flow cytometry. Parental THP-1 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>
FCGR2A (Human) CRISPR/Cas9 Lentivirus (Integrating)	78207	500 μ l x 2
FcGR2B– CHO K1 Recombinant Cell Line	79511	2 vials
FCGR2A, Avi-His-Tag	100089	100 μ g
FCGR2B, Avi-His-Tag	100094	100 μ g
FCGR2B, Avi-His-Tag, Biotin-labeled	100474-1	25 μ g