

Description

Fc Gamma Receptor 2A (also known as CD32A, Fc-gamma RIIa, FcγRIIa) is a low affinity Fc receptor for immunoglobulin G, encoded by the FCGR2A gene. Fc Gamma Receptor 2A is a cell surface receptor that is expressed on a variety of immune cells such as macrophages and neutrophils. It is involved in phagocytosis and in the clearing of spent immune complexes from the circulation. A polymorphism in FCGR2A has been associated with increased risks of nephritis and lupus.

The FCGR2A CRISPR/Cas9 Lentiviruses are replication incompetent, HIV-based VSV-G pseudotyped lentiviral particles that are ready to infect most 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 (Figure 1 and Table 1).

The non-integrating lentivirus is made with a mutated integrase, resulting in only transient expression of Cas9 and sgRNA. Although using the non-integrating lentivirus results in lower knockdown efficiency, Cas9 is not permanently expressed, which lowers the risk of off-targeting, and there are no random integrations into the cell’s genome. Despite transient expression of Cas9 and sgRNA, knockout cell lines can still be generated using cell sorting or limiting dilution due to the permanent changes in the genomic DNA from the Cas9 nuclease activity and NHEJ repair.

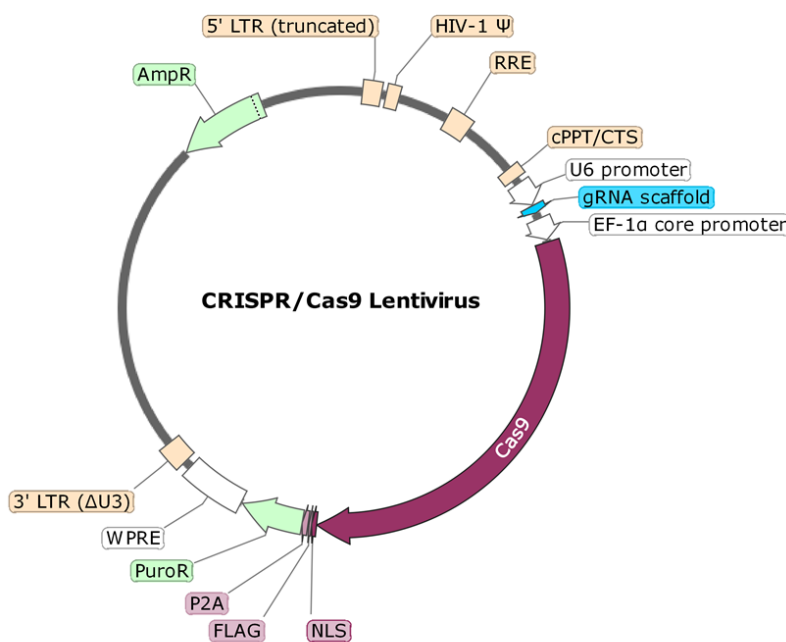


Figure 1: Schematic of the lenti-vector used to generate the FCGR2A CRISPR/Cas9 Lentivirus.

Gene Target:	sgRNA Sequence:
FCGR2A-1-F	TGGAGCACGTTGATCCACGG
FCGR2A -2-F	AGGGAGAAACCATCATGCTG
FCGR2A -3-F	GCTTGTGGGATGGAGAAGGT
FCGR2A -4-F	AGCAGCAGCAAAACTGTCAA
FCGR2A -5-F	AAAGCACAGTCAGATGCACA

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

Application

- Transient knockdown of FCGR2A in target cells
- Generation of a stable FCGR2A knockout cell line following transient puromycin selection (48 hours maximum) and limiting dilution

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 μ l x 2) of lentivirus at a titer $\geq 1 \times 10^7$ 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



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.

Validation Data

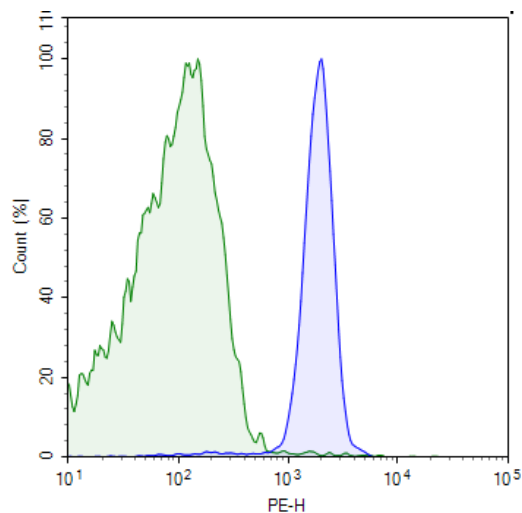


Figure 3: Knockdown of FCGR2A in Jurkat cells using FCGR2A CRISPR/Cas9 Lentivirus.

Jurkat cells were transduced via spinoculation with 1×10^7 TU/well of FCGR2A CRISPR/Cas9 lentivirus, corresponding to an MOI >5 . 48 hours after transduction, cells were stained with CoraLite 488-conjugated FCGR2A antibody (ThermoFisher #CL488-66529) 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.

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>
CTLA4 CRISPR/Cas9 Lentivirus (Non-Integrating)	78061	500 µl x 2
CTLA4 CRISPR/Cas9 Lentivirus (Integrating)	78054	500 µl x 2
TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)	78065	500 µl x 2
TIGIT CRISPR/Cas9 Lentivirus (Integrating)	78058	500 µl x 2
CD47 CRISPR/Cas9 Lentivirus (Non-Integrating)	78063	500 µl x 2
CD47 CRISPR/Cas9 Lentivirus (Integrating)	78056	500 µl x 2