

Description

FcγRI (CD64) Lentivirus are replication incompetent, HIV based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. These particles contain the FcγRI (Fc-gamma receptor 1), also known as CD64, under the control of an EF1a promoter. The lentiviruses also transduce a hygromycin selection marker (Figure 1).

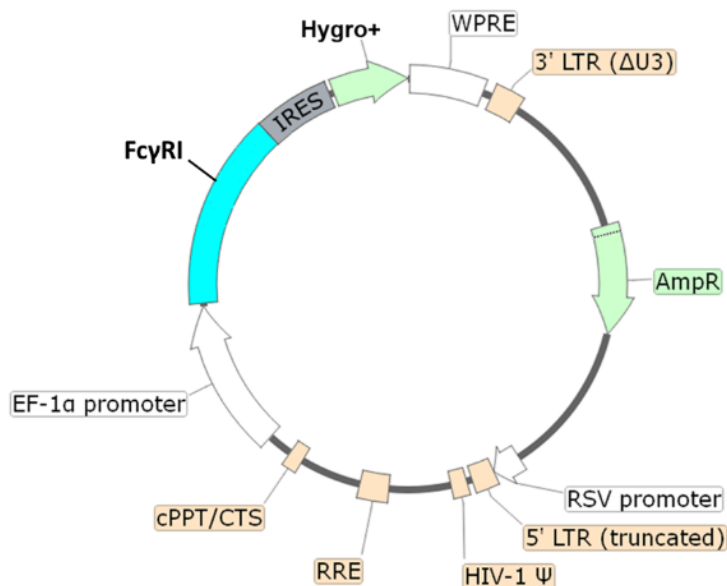


Figure 1. Schematic of the lenti-vector used to generate the FcγRI (CD64) Lentivirus.

Background

Antibody-dependent cell-mediated phagocytosis (ADCP) is an important mechanism to consider during antibody drug development. FcγRI (Fc gamma receptor 1), also known as CD64, is found in macrophages and monocytes, and its expression can be induced in polymorphonuclear leukocytes by IFNγ (interferon gamma) and G-CSF (granulocyte colony-stimulating factor). Multivalent IgG immune complexes (ICs) promote receptor clustering and activation of intracellular signaling through phosphorylation of ITAMs (immunoreceptor tyrosine-based activation motifs), leading to phagocytosis via Rac-GEFs (guanine exchange factors). This mechanism of action has contributed to the success of rituximab (anti-CD20 antibody) in the treatment of chronic lymphocytic leukemia (CLL). However, an overactivation of FcγRI can result in chronic inflammation and autoimmune disorders, such as SLE (systemic lupus erythematosus), ITP (immune thrombocytopenia) and RA (rheumatoid arthritis). Leveraging FcγRI properties can improve cancer therapy, while blocking its activation is a promising therapeutic approach for autoimmune disorders.

Application(s)

- Generation of cell pools or stable cell lines expressing FcγRI (CD64) following hygromycin selection.

Formulation

The lentivirus particles were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentivirus at a titer $\geq 10^7$ 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 for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. 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.

Notes

To generate an FcγRI expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of hygromycin as pre-determined from a killing curve, <https://bpsbioscience.com/kill-curve-protocol>, for antibiotic selection of transduced cells, followed by clonal selection.

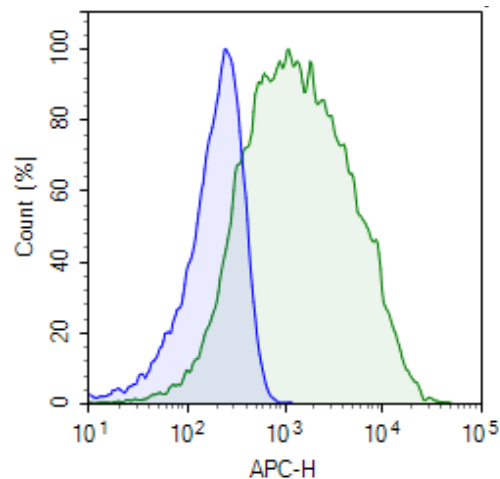
Figures and Validation Data

Figure 2. Expression of FcGRI (CD64) in Jurkat cells transduced with FcGRI (CD64) Lentivirus by flow cytometry.

Approximately 200,000/well of NFAT Luciferase reporter Jurkat cells (#60621) were transduced with 1,000,000 TU/well of FcGRI (CD64) Lentivirus. 66 hours post-transduction, cells were selected in Thaw medium 2 (#60184) plus 200 µg/ml of hygromycin for ~10 days. The hygromycin-resistant cell pool was analyzed by flow cytometry using an APC-conjugated anti-CD64 antibody. Y-axis represents the cell count. X-axis indicates APC intensity.

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

Version 052126