

Description

HLA-C*08:02 Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These viruses result in expression of human HLA-C*08:02 driven by an EF1a promoter and a puromycin selection marker (Figure 1).

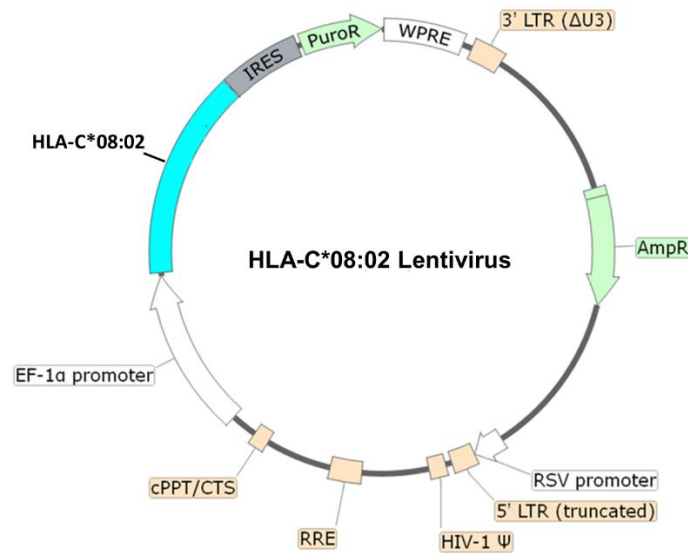


Figure 1. Schematic of the lenti-vector used to generate HLA-C*08:02 Lentivirus.

Background

Human Leukocyte Antigen-C (HLA-C) are MHC (major histocompatibility complex) I heavy chain receptor, composed of HLA-C and β 2-microglobulin (B2M). HLA-C is present in all cells and exists as several haplotypes due to the diversity of HLA-C genes. C*08:02 represents one such haplotype. HLA class I present neoantigen derived peptides to the cell surface, allowing them to be recognized by T cells, via TCR (T cell receptors). Cancer immunotherapy has been taking advantage of that mechanism, by engineering T cells to express TCRs able to recognize specific cancer immunogens. In 2016 the use of HLA-C*08:02-restricted TIL (tumor infiltrating lymphocytes) targeting specifically KRAS (Kirsten rat sarcoma virus) G12D mutation in lung cancer resulted in positive results. Last year a similar approach was pursued in a patient with metastatic pancreatic cancer and resulted in regression of the disease. The study of HLA-C*08:02-restricted TIL expressing TCR against other neoantigens may prove beneficial in cancer therapy.

Application(s)

- Expression of human HLA-C*08:02 in cells of interest.
- Generate cell pools or stable cell lines expressing HLA-C*08:02 following puromycin 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 μ l 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 . Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

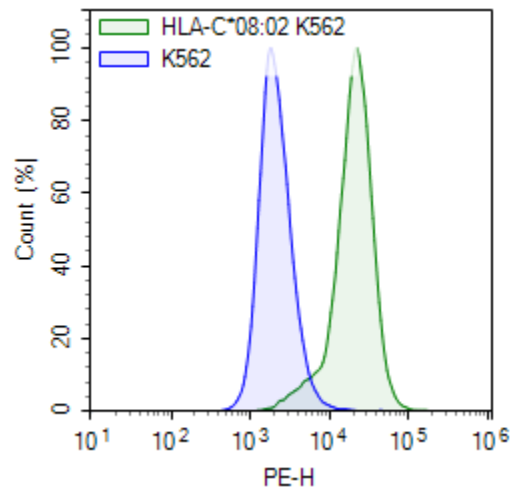


The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they 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 HLA-C*08:02 expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, <https://bpsbioscience.com/cell-line-faq>), for antibiotic selection of transduced cells, following by clonal selection.

Figures and Validation Data



*Figure 2. Expression of HLA-C*08:02 in K562 cells transduced with HLA-C*08:02 Lentivirus.*

Approximately 100,000 K562 cells were transduced with 1×10^6 TU ($100 \mu\text{l}$ of 10^7 TU/ml) of HLA-C*08:02 Lentivirus via spinoculation ($800 \times g$ at 32°C for 30 minutes) in the presence of $5 \mu\text{g}/\text{ml}$ of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (BPS Bioscience #78939). 48 hours post-transduction, the cells were cultured with $1 \mu\text{g}/\text{ml}$ of puromycin. The puromycin-resistant cell pool was stained with HLA-C Polyclonal Antibody (Thermo Fisher #PA5-79367) followed by PE Donkey anti-rabbit IgG (minimal x-reactivity) Antibody (Biolegend #406421) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Sequence

Human HLA-C*08:02 sequence

MRVMAPRTLILLLSGALALTETWACSHSMRYFYTAVSRPGRGEPRIAVGVYDDTQFVQFSDAASPRGEPRAPWVEQEGPEY
 WDRETQKYKRQAQTDRVSLRNLRGYNQSEAGSHTLQRMYGCDLGPDRLLRGYNQFAYDGDYIALNEDLRSWTAADKAA
 QITQRKWEAAREAEQRRAYLEGTCVEWLRRYLENGKKTQRAEHPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGED
 QTQDTELVETRPAGDGTQKWA AVVVPSGEEQRYTCHVQHEGLPEPLTRWGPSSQPTIPIVGIVAGLAVLAVLAVLGAVMAV
 VMCRKSSGGKGGSCSAASSNSAQGSDESLIACKA

ReferencesLeidner R., *et al.*, 2022 *N Engl J Med* 386:2112-2119Tran E., *et al.*, 2016 *N Engl J Med* 375:2255-2262.**Troubleshooting Guide**

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

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Firefly Luciferase-eGFP Lentivirus (G418 or Puromycin)	79980	500 µl x 2
Expression Negative Control Lentivirus (EF1A Promoter/ Puromycin)	82212-P	500 µl x 2
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	78939	500 µl
Anti-HLA-DR Biotin-Labeled Antibody	101769	100 µg
HLA-E Lentivirus	78929	500 µl x 2

Version 011524