Description

HLA-E 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 the human HLA-E heavy chain (NM_005516.6) driven by an EF1a promoter, and a puromycin selection marker (Figure 1).

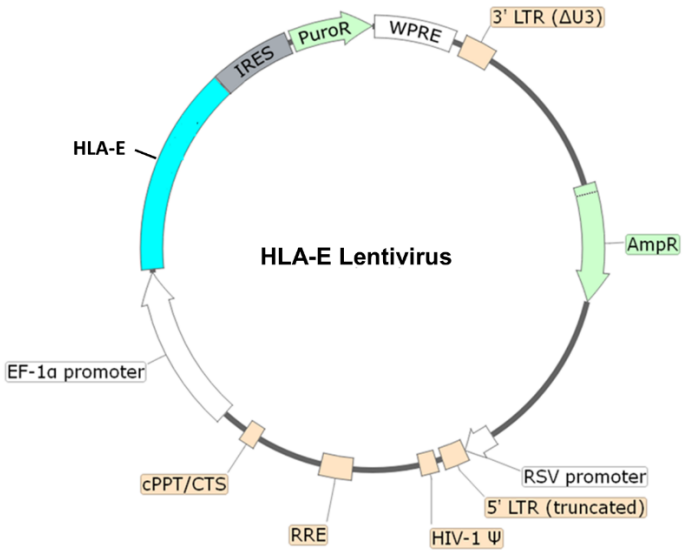

Figure 1. Schematic of the lenti-vector used to generate HLA-E Lentivirus (encoding HLA-E heavy chain).

Background

HLA-E, or MHC (major histocompatibility complex) class I antigen E, is considered a non-classical MHC class I with low expression and fewer polymorphisms than the remaining HLA. HLA-E is composed of a heavy chain and β-2 microglobulin (B2M). It binds to specific peptides derived from the classical MHC class I (HLA-A, B, C and G), after these have been processed in the endoplasmic reticulum and the proteosome. The complex of HLA-E with the peptide is recognized by NK cells via the inhibitory receptor CD94/NKG2A/B. Binding to CD94/NKG2C however results in NK cell activation. Expression of HLA-E combined with knockout of HLA-A, B and C, in pluripotent stem cell (PSC) and their differentiated cell types, resulted in these cells escaping attack by $CD8^+$ T cells and NK cytotoxicity. This strategy brings us closer to an almost universal cell donor reality, reducing the risk of immune rejection during cell transplants and alleviating the enormous investment of creating a PSC bank that has representation of all the haplotypes.

Application(s)

- Expression of human HLA-E in cells of interest.
- Generate cell pools or stable cell lines expressing HLA-E 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 and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥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-E 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, followed by clonal selection.

Figures and Validation Data

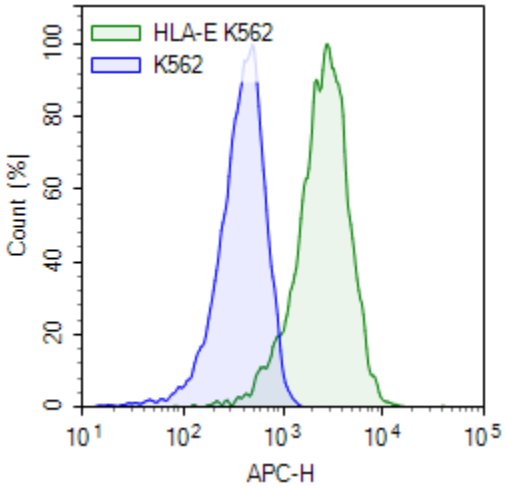

Figure 2. Expression of HLA-E in K562 cells transduced with HLA-E Lentivirus.
Approximately 100,000 K562 cells were transduced with 1 x 10^6 TU (100 µl of 10^7 TU/ml) of HLA-E Lentivirus via spinoculation (800 x *g* at 32°C for 30 minutes) in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (BPS Bioscience #78939). 48 hours post-transduction, the cells were cultured with 1 µg/ml of puromycin. The puromycin-resistant cell pool was stained with APC anti-human HLA-E Antibody (Biolegend #342605) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates APC intensity.

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

Sequence
Human HLA-E sequence (accession number NM_005516.6)

MVDGTLLLLLSEALALTQTWAGSHSLKYFHTSVSRPGRGEPRFISVGYVDDTQFVRFDNDAASPRMVPRAPWMEQEGSEYWD
RETRSARDTAQIFRVNLRTLRGYYNQSEAGSHTLQWMHGCELGPDGRFLRGYEQFAYDGKDYLTLNEDLRSWTAVDTAAQISE
QKSNDASEAEHQRAYLEDTCVEWLHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDT
ELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPIVGIIAGLVLLGSVVSGAVVAAVIWRKKSS
GGKGGSYSKAEWSDSAQGSESHSL

References
Gornalusse G., *et al.*, 2017 *Nature Biotechnology* 35:765-772.

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

Related Products

Products	*Catalog #*	*Size*
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-C*08:02 Lentivirus	78930	500 µl x 2

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