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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

B2M HLA-E Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce most mammalian cells, including primary and non-dividing cells. These viruses result in the expression of B2M (beta-2 microglobulin) HLA (human leukocyte antigen)-E driven by an EF1a promoter. The lentiviruses also contain a puromycin selection marker (Figure 1).

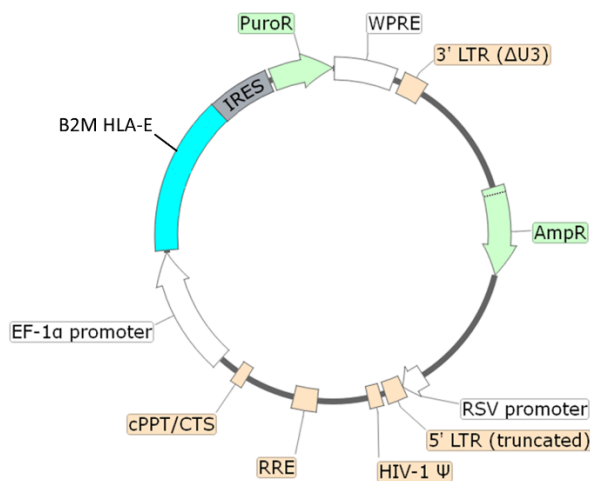


Figure 1. Schematic of the lenti-vector used to generate B2M HLA-E Lentivirus.

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 proteasome. 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 B2M HLA-E in cells of interest.
- Generate B2M HLA-E expressing cell pools or stable cell lines by puromycin selection.

Formulation

The lentivirus particles were produced in HEK293T cells. They are supplied 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 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 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 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/kill-curve-protocol>), for antibiotic selection of transduced cells, followed by clonal selection.

Validation Data

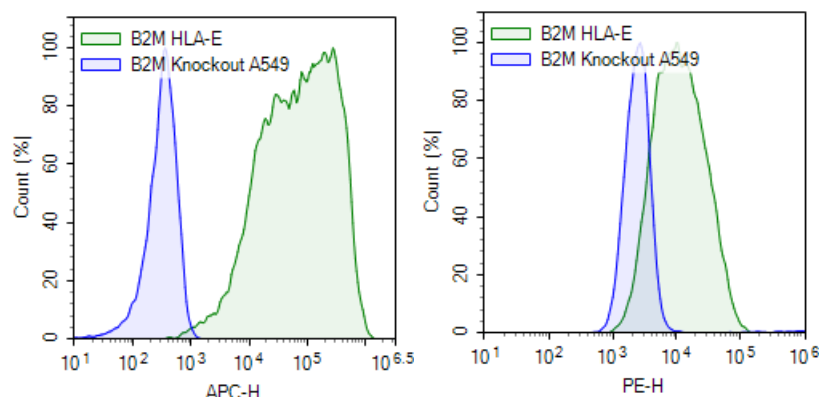


Figure 2. Expression of B2M and HLA-E in B2M Knockout A549 cells transduced with B2M HLA-E Lentivirus.

Approximately 100,000 B2M Knockout A549 cells (#82871) were transduced with 1×10^6 TU (100 μ l 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 μ g/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (#78939). 48 hours post-transduction, the cells were stained with (A) APC anti-human HLA-E Antibody (BioLegend #342605) and (B) PE anti-human β 2-microglobulin Antibody (BioLegend #395704) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates APC or PE intensity.

Data is representative.

References

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

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>.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
HLA-A*01:01 Lentivirus	82423	500 µl x 2
B2M Knockout A549 Cell Line	82871	2 vials
HLA-C*08:02 K562 Cell Line	78974	2 vials
HLA-C*08:02 Lentivirus	78930	500 µl x 2
HLA-A/B/C Knockout Electroporation Kit	82395	1 Kit
HLA-A/B/C Knockout HEK293T Cell Line	82943	2 vials

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