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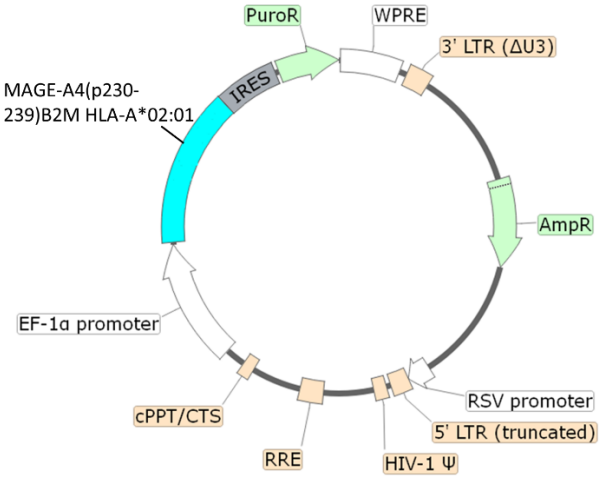
Description

MAGE-A4 (p230-239) B2M HLA-A*02:01 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 MAGE-A4 (melanoma associated antigen A4) peptide 230-239, B2M (beta-2 microglobulin), and HLA-A (human leukocyte antigen) *02:01. The lentiviruses also contain a puromycin selection marker (Figure 1).

A



B

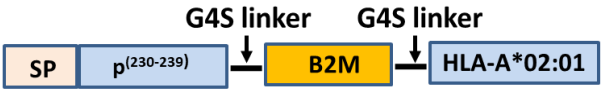


*Figure 1. (A) Schematic of the lenti-vector used to generate MAGE-A4 (p230-239) B2M HLA-A*02:01 Lentivirus. (B) Construct diagram showing components of the MAGE-A4 (p230-239) B2M HLA-A*02:01.*

Background

Human Leukocyte Antigen-A (HLA-A) is an MHC-I (major histocompatibility complex) heavy chain receptor, composed of HLA-A and β2-microglobulin (B2M). There are over 200 genes encoding HLA variants and this variability plays a critical role in adaptive immunity. HLA-A*02 is one of the most common class I types. HLA class I are involved in presenting peptides that are typically between 8 to 11 amino acids. HLA-A*02:01 can bind 15-mer peptides, which can then be recognized by T cells. Studies in SCLC (small cell lung cancer) patients has shown that an ATAD2 (ATPase family AAA domain-containing protein 2) immunopeptide can be used in HLA-A*02-01-restricted patients with high reactivity.

MAGE (melanoma associated antigen) proteins are CT (cancer testis) antigens, and there are about 60 proteins in the MAGE family that can be subdivided into type I (present only on the X-chromosome, MAGE-A, B and C) and type II (MAGE D-L and necdin). Under normal conditions they are mostly found in the testis and placenta. They are found at high levels in several cancer types, such as melanoma, brain, and breast cancer, and are involved in the development of resistance to chemotherapy, cell motility and cell survival. Expression of MAGE proteins tend to correlate with a poor prognosis. They are intracellular proteins, with MAGE-A4 being found in the cytosol and nucleus, making them poor targets for strategies such as CAR-T cell therapy. MAGE proteins are degraded in the proteosome, and the peptides created can then be found on the cell membrane in combination with MHC (major

histocompatibility complex) I. The presentation on the cell surface in this form makes them an attractive target for TCR (T cell receptor)-T cell therapy. In 2024, the first MAGE-A4 TCR engineered cell therapy for advanced synovial sarcoma was approved by the Food and Drug Administration (FDA).

Application(s)

- Expression of human MAGE-A4 (p230-239) B2M HLA-A*02:01 in cells of interest.
- Generate MAGE-A4 (p230-239) B2M HLA-A*02:01 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 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 for up to 12 months from date of receipt. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

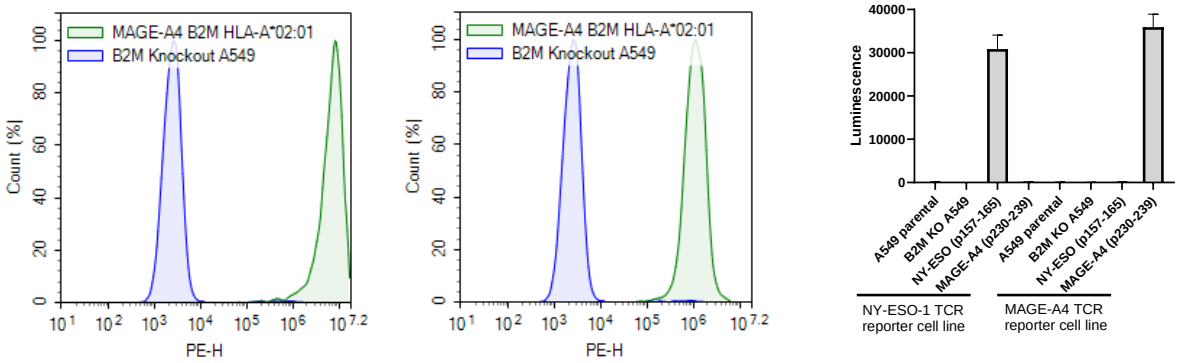
Biosafety

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 a MAGE-A4 (p230-239) B2M HLA-A*02:01 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



A B C

*Figure 2. Expression and function of MAGE-A4 (p230-239) B2M HLA-A*02:01 in B2M Knockout A549 cells transduced with MAGE-A4 (p230-239) B2M HLA-A*02:01 Lentivirus.*
Approximately 100,000 B2M Knockout A549 cells (#82871) were transduced with 1 x 10^6 TU (100 µl of 10^7 TU/ml) of MAGE-A4(p230-239) B2M HLA-A*02:01 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). **(A)** 48 hours post-transduction, the cells were stained with PE anti-human HLA-A2 Antibody (BioLegend #343306) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity. **(B)** Cells were also stained with PE anti-human β2-microglobulin Antibody (BioLegend #395704) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity. **(C)** 72 hours post-transduction, the cells were seeded into a 96-well plate (20,000 cells/well) overnight, and co-cultured with NY-ESO-1 TCR CD8+ NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78769) or MAGE-A4 TCR CD8+ NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78984) overnight. Luciferase activity was measured with ONE-Step™ Luciferase Assay System (BPS Bioscience #60690), and the results are shown as raw luminescence readings. A549 parental cells and B2M Knockout A549 cells (BPS Bioscience #82871) were run in parallel as negative controls. MAGE-A4 TCR CD8+ NFAT Luciferase Reporter Jurkat cells can be activated by cells transduced with MAGE-A4 (p230-239) B2M HLA*A02:01 Lentivirus, but not by cells transduced with NY-ESO-1 (p157-165) B2M HLA*A02:01 Lentivirus (BPS Bioscience #82440).

Data is representative.

References

Kropp KN., *et al.*, 2020 *PLOS One* 15(9): e0238875.
Caballero, O L.., *et al.*, 2009 Cancer Sci. 100, 2014–2021.
Sanderson, J. P. et al., 2020 Oncoimmunology 9, 1682381.
Hassan C., *et al.*, 2014 *J Biol Chem* 290(5):2593-2603.
Yuan L., *et al.*, 2025 *eBioMedicine* 112: 105515.

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

Products	*Catalog #*	*Size*
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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