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Description

NY-ESO-1 (p157-165) 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 NY-ESO-1 (New York esophageal squamous cell carcinoma 1) peptide 157-165, B2M (beta-2 microglobulin), and HLA (human leukocyte antigen)-A*02:01. The lentiviruses also contain a puromycin selection marker (Figure 1).

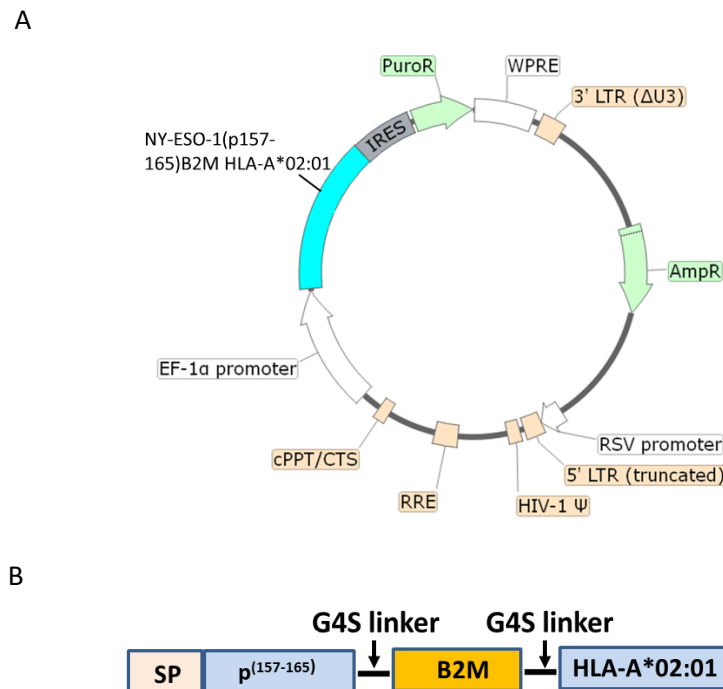


Figure 1. (A) Schematic of the lenti-vector used to generate NY-ESO-1 (p157-165) B2M HLA-A*02:01 Lentivirus. (B) Construct diagram showing components of the NY-ESO-1 (p157-165) B2M HLA-A*02:01.

Background

Human Leukocyte Antigen-A (HLA-A) is an MHC-I (major histocompatibility complex) heavy chain receptor, composed of HLA-A and β 2-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.

NY-ESO-1 (New York esophageal squamous cell carcinoma 1, also known as Cancer/testis antigen 1, or CTAG1B), is an important tumorigenic marker present in malignant cells. Normally expressed only in embryonic testis, this highly immunogenic protein is not usually found in normal tissues, but is re-expressed in multiple myeloma, non-small cell lung carcinoma, and breast and ovarian cancer, making it a promising candidate antigen for cancer immunotherapy. Several NY-ESO-1-directed therapies are being developed including cancer vaccines, anti-NY-ESO-1 adoptive cell therapy, alone or in combination with checkpoint inhibitors.

Application(s)

- Expression of human NY-ESO-1 (p157-165) B2M HLA-A*02:01 in cells of interest.
- Generate NY-ESO-1 (p157-165) 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 $\geq 10^7$ 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 NY-ESO-1 (p157-165) 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

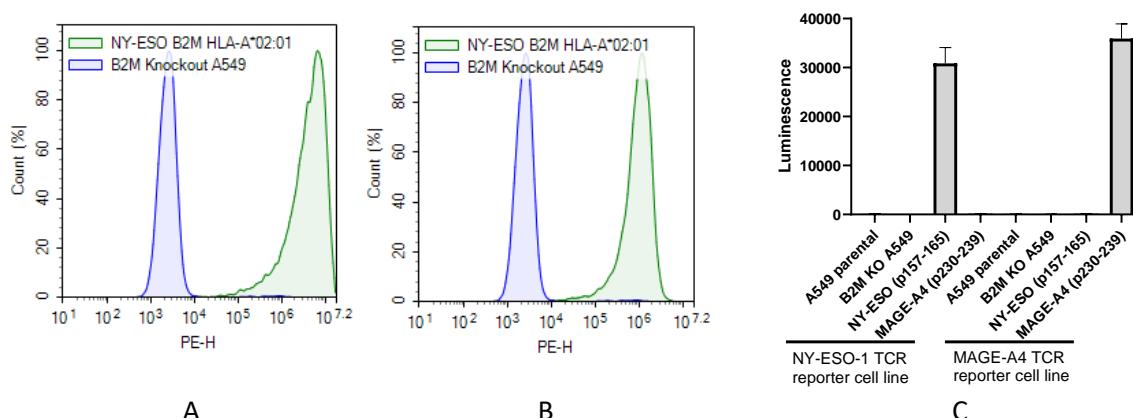


Figure 2. Expression and function of NY-ESO-1(p157-165) B2M HLA-A*02:01 in B2M Knockout A549 cells transduced with NY-ESO-1 (p157-165) B2M HLA-A*02:01 Lentivirus.

Approximately 100,000 B2M Knockout A549 cells (#82871) were transduced with 1×10^6 TU (100 μ l of 10^7 TU/ml) of NY-ESO-1(p157-165) B2M HLA-A*02:01 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 (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 β 2-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. NY-ESO-1 TCR CD8+ NFAT Luciferase Reporter Jurkat cells can be activated by cells transduced with NY-ESO-1 (p157-165) B2M HLA-A*02:01 Lentivirus, but not by cells transduced with MAGE-A4 (p230-239) B2M HLA-A*02:01 Lentivirus (BPS Bioscience #82441).

Data is representative.

References

- Thomas R., *et al.*, 2018 *Front. Immunol.* 9: 00947.
 Raza A., *et al.*, 2020 *J. of Translational Medicine* 140.
 Kropp KN., *et al.*, 2020 *PLOS One* 15(9): e0238875.
 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

<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

Version 061825