SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The HLA-A/B/C Knockout HEK293T Cell Line is a clonal HEK293T cell line that does not express Human Leukocyte Antigen (HLA)-A, HLA-B, and HLA-C. This knockout cell line was generated by CRISPR/Cas9 genome editing using sgRNAs that target regions common to all three genes.

This cell line has been validated by flow cytometry to confirm the knockout, as well as HLA-A, B, and C rescue experiments.

Background

HLA (Human Leukocyte Antigens)-A, B, and C are the three major types of MHC (major histocompatibility complex) class 1 transmembrane proteins. They form a heterodimer with the β2 microglobulin protein (encoded by the B2M gene). The MHC class 1 molecules present short polypeptides, usually between 7-11 amino acids long, to the immune system for recognition as either "self" or "non-self". HLA-C, for instance, 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 this 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) specifically targeting KRAS (Kirsten rat sarcoma virus) G12D mutation in lung cancer led to positive results. 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. HLA mismatching between donor cells and the individual can lead to immune rejection, and one option is the knockout of the endogenous HLA, allowing cells to be universally used. The use of a cell line, in which the three major MHC class 1 proteins are not expressed, can be used a cellular model that can be used to express specific HLA alleles.

Application

- Useful for expressing specific HLA-A, B, or C alleles for antigen presentation.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293T, human epithelial cell line, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 6	BPS Bioscience #60183

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. Cells should be grown at 37˚C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 6.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 6.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. Change medium to fresh Thaw Medium 6 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Thaw Medium 6.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium, and resuspend the cells in Thaw Medium 6.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for storage for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

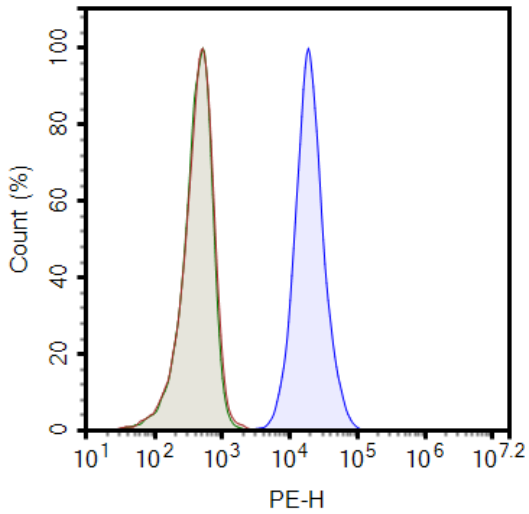

Figure 1. Expression of HLA in the HLA-A/B/C Knockout HEK293T Cell Line.
HLA-A/B/C Knockout HEK293T cells (red) or parental HEK293T cells (blue) were stained with PE-anti-human HLA-A,B,C Antibody (BioLegend #311406) and analyzed by flow cytometry. Unstained parental HEK293T cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

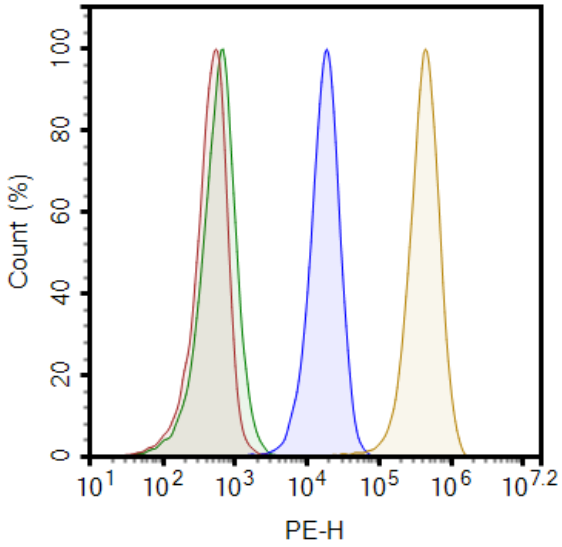

Figure 2. Re-expression of an HLA-A allele in the HLA-A/B/C Knockout HEK293T Cell Line.
HLA-A/B/C Knockout HEK293T cells (red) were transduced with a lentiviral vector carrying the cDNA encoding HLA-A*02:01 (#82424) (yellow). Parental HEK293T cells are shown in blue. Cells were stained with PE anti-human HLA-A2 Antibody (BioLegend #343306) and analyzed by flow cytometry. Unstained parental HEK293T cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

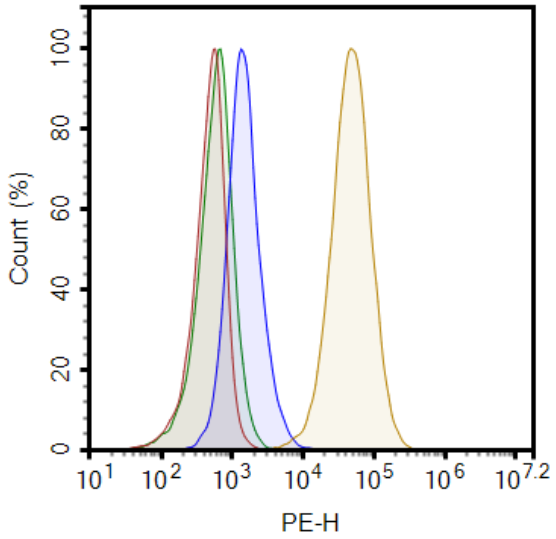

Figure 3. Re-expression of an HLA-B allele in the HLA-A/B/C Knockout HEK293T Cell Line.
HLA-A/B/C Knockout HEK293T cells (red) were transduced with a lentiviral vector carrying the cDNA encoding HLA-B*07:02 (#82428) (yellow). Parental HEK293T cells are shown in blue. Cells were stained with PE anti-human HLA-B7 Antibody (BioLegend #372404) and analyzed by flow cytometry. Unstained parental HEK293T cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

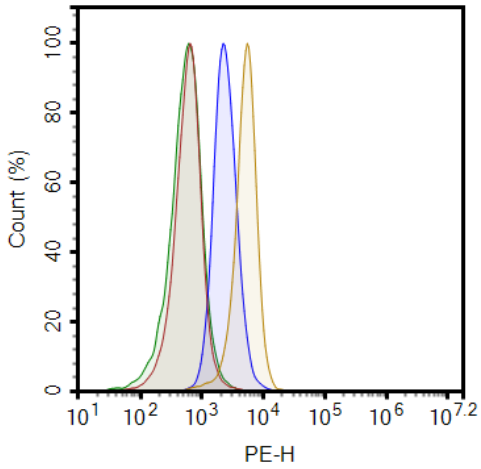

Figure 4. Re-expression of an HLA-C allele in the HLA-A/B/C Knockout HEK293T Cell Line. HLA-A/B/C Knockout HEK293T cells (red) were transduced with a lentiviral vector carrying the cDNA encoding HLA-C*8:02 (#78930) (yellow). Parental HEK293T cells are shown in blue. Cells were stained with HLA-C Polyclonal Antibody (ThermoFisher #PA5-79367) followed by PE Donkey anti-rabbit IgG Antibody (BioLegend #406421) and analyzed by flow cytometry. Unstained parental HEK293T cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

Data shown is representative.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting

For lot-specific information and all other questions, please email visit https://bpsbioscience.com/contact. Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions.

Notes

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

References

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

Related Products

Products	*Catalog #*	*Size*
HLA-A/B/C Knockout Electroporation Kit	82395	1 kit
B2M HLA-A*02:01 Lentivirus	82424	500 µl x 2
B2M HLA-B*07:02 Lentivirus	82428	500 µl x 2
HLA-C*08:02 Lentivirus	78930	500 µl x 2

Version 041625