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Produktinformation

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

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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-C*08:02 K562 Cell Line is an engineered human lymphoblast K562 cell line expressing human HLA-C*08:02 driven by an EF1a promoter. This cell line was generated by transduction with HLA-C*08:02 Lentivirus (#78930).

This cell line has been validated by flow cytometry and co-culture assays with Jurkat cells expressing KRAS G12D, in the presence of wild type and mutant KRAS peptides.

Background

Human Leukocyte Antigen-C (HLA-C) is an MHC-I (major histocompatibility complex) heavy chain receptor, composed of HLA-C and β2-microglobulin (B2M). HLA-C 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 that 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) targeting specifically KRAS (Kirsten rat sarcoma virus) G12D mutation in lung cancer resulted in 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. K562 cells are HLA class I and II negative, making them an ideal cellular model to introduce and study specific haplotype responses.

Application

- Useful for studying antigen presentation by HLA-C.
- Useful for studying HLA-C restricted T cell responses.

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

K562, human lymphoblast cell line, suspension.

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 2	BPS Bioscience #60184
Growth Medium 2E	BPS Bioscience #79638

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. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. 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 2 (BPS Bioscience #60184):
RPMI-1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2E (BPS Bioscience #79638):
RPMI-1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 µg/ml of puromycin.

Cell Culture Protocol

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 content of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2.

   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 2.

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. For a T25 flask, add 3-4 ml of Thaw Medium 2, and continue growing in a 5% CO2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. Switch to Growth Medium 2E at first and subsequent passages.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, with Growth Medium 2E. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. 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.

2. 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.

3. Transfer the vials to liquid nitrogen the next day 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.

Validation Data

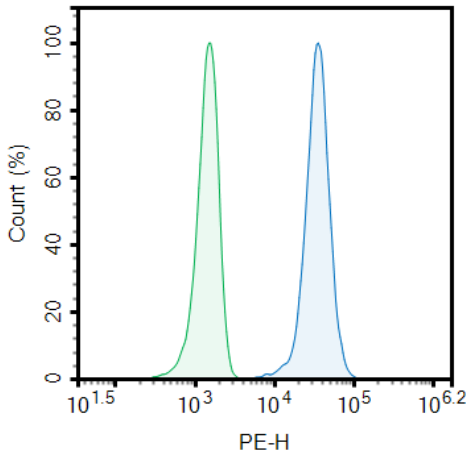

*Figure 1: Cell surface expression of HLA-C*08:02 in HLA-C*08:02 K562 Cell Line by flow cytometry.* HLA-C*08:02 K562 cells and control parental K562 cells were stained with HLA-C Polyclonal Antibody (Thermo Fisher #PA5-79367) followed by PE Donkey anti-rabbit IgG Antibody (Biolegend #406421) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity.

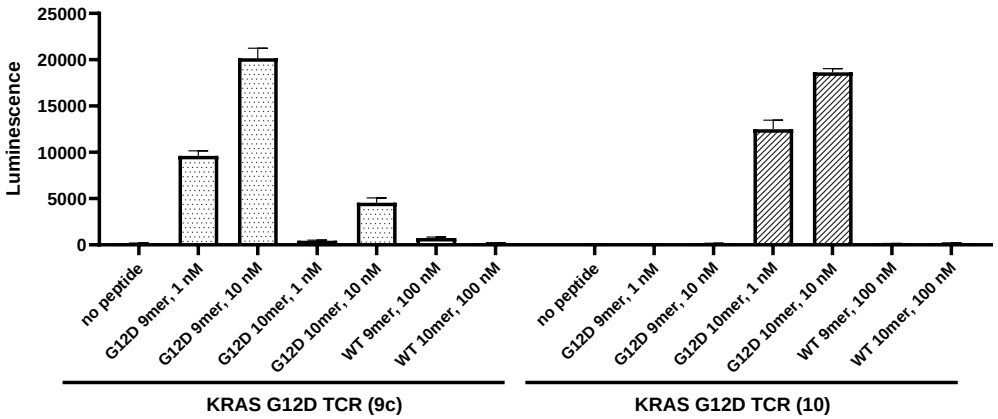

*Figure 2. Jurkat T cell activation after transduction with KRAS G12D-Specific TCR Lentivirus, using HLA-C*08:02 K562 Cell Line as antigen presenting cells (APCs).*
$CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (#78757) were transduced with KRAS G12D TCR Specific Lentivirus (Clone 9c) (#78936) or KRAS G12D TCR Specific Lentivirus (Clone 10) (#78937) by spinoculation at a MOI of 10. Sixty-six hours post-transduction, the cells were co-cultured for 6 hours with HLA-C*08:02 K562 cells loaded with KRAS G12D Peptide 9mer (10-18, #78967), KRAS WT Peptide 9mer (10-18, #78968), KRAS G12D Peptide 10mer (10-19, #78969) or KRAS WT Peptide 10mer (10-19, #78970). Luciferase activity was measured, and the results are shown as raw luminescence readings. Cells transduced with KRAS G12D Specific TCR Lentivirus (Clone 9c) are preferentially activated by KRAS K12D Peptide 9mer, while cells transduced with KRAS G12D Specific TCR Lentivirus (Clone 10) are preferentially activated by KRAS K12D Peptide 10mer. Both KRAS G12D TCR (Clone 9c) and KRAS G12D TCR (Clone 10) transduced cells are specific for mutant KRAS G12D peptides and did not recognize wild type KRAS peptides.

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

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

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-C*08:02 Lentivirus	78930	500 µL x 2
KRAS G12D TCR Specific Lentivirus (Clone 9c)	78936	500 µL x 2
KRAS G12D TCR Specific Lentivirus (Clone 10)	78937	500 µL x 2

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