Description

The HLA-B*08:01 A549 Cell Line is an A549 human lung cancer cell line generated by selecting a single clone of B2M Knockout A549 cells (#82871) transduced with a lentivirus expressing B2M (beta-2 microglobulin) HLA (human leukocyte antigen)-B*08:01 driven by an EF1a promoter (#82429).

This cell line has been validated by flow cytometry.

Background

Human Leukocyte Antigen-B (HLA-B) is an MHC-I (major histocompatibility complex) heavy chain receptor, composed of HLA-B and β2-microglobulin (B2M). There are over 200 genes encoding HLA variants and this variability plays a critical role in adaptive immunity. HLA-B has been linked to autoimmune disorders and drug reactions. HLA-B*08 has been linked to anti-carbamylated protein antibody-positive/anti-cyclic citrullinated peptide-negative RA (rheumatoid arthritis). HLA-B*08:01 has been identified as a unique genetic factor in EOMG (early-onset myasthenia graves). The understanding of the association between HLA alleles and diseases will allow a refinement of therapeutic strategies.

Application

- Use as a control for studying the differences between HLA-B alleles.
- Study T cell activation and responses to peptides presented by HLA-B*08:01.

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

A549 is a human lung alveolar basal carcinoma cell line. Adherent epithelial cells.

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
Growth Medium 6C	BPS Bioscience #78077

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℃ 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.

Growth Medium 6C (BPS Bioscience #78077):

DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 0.25 µg/ml puromycin.

Cell Culture Protocol

Note: A549 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 attachment and 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. Replace media every 2-3 days until cells reach 90% confluency. At first passage and subsequent passages, use Growth Medium 6C.

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 0.25% 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 Growth Medium 6C .

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 once or 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 0.25% Trypsin/EDTA.

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

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~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 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

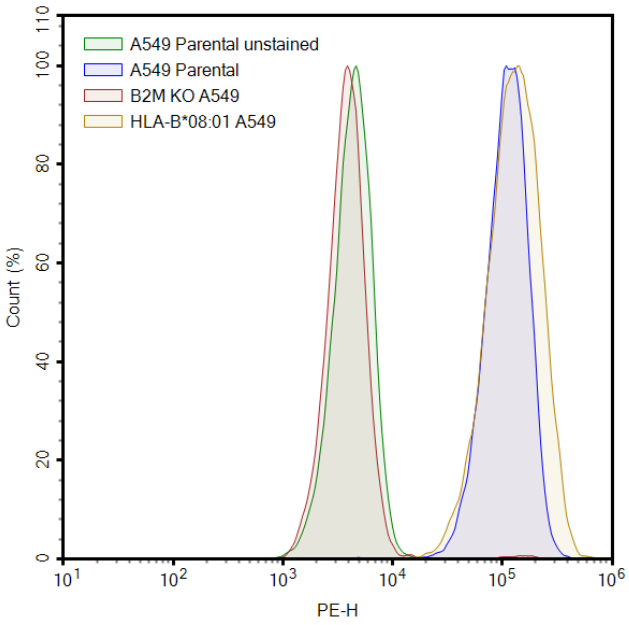

*Figure 1. Expression of HLA-B*08:01 in the HLA-B*08:01 A549 Cell Line.*
Parental A549 cells (blue), B2M Knockout A549 cells (BPS Bioscience #82871, red), and HLA-B*08:01 A549 cells (yellow) were stained with PE anti-human HLA-A/B/C Antibody (BioLegend #311405) and analyzed by flow cytometry. Unstained parental A549 cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

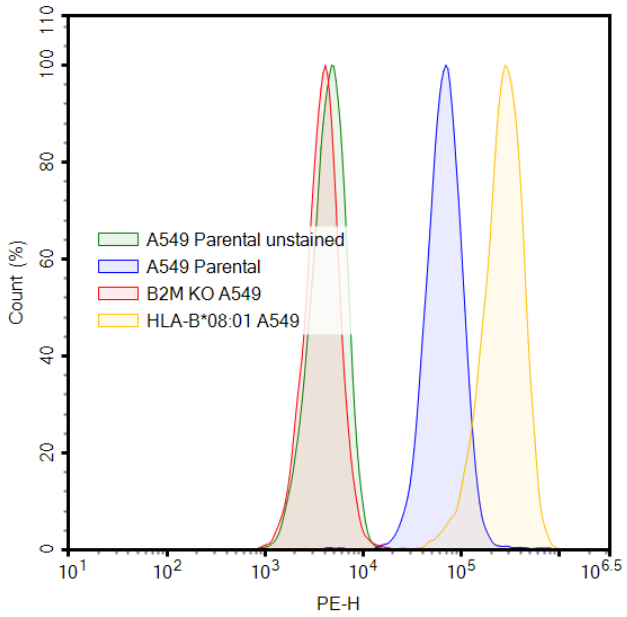

*Figure 2. Expression of B2M-HLA-B*08:01 in the HLA-B*08:01 A549 Cell Line.*
Parental A549 cells (blue), B2M Knockout A549 cells (BPS Bioscience #82871, red), and HLA-B*08:01 A549 cells (yellow) were stained with PE anti-human β2-microglobulin Antibody (BioLegend #395703) and analyzed by flow cytometry. Unstained parental A549 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. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Troubleshooting Guide
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.

Related Products

Products	*Catalog #*	*Size*
B2M Knockout Jurkat Cell Line	82872	2 vials
B2M Knockout THP-1 Cell Line	78389	2 vials
B2M Knockout iPS Cell Line	82161	1 vial
B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line	78363	2 vials
B2M (Human) CRISPR/Cas9 Lentivirus (Integrating)	78340	500 µl x 2
B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78341	500 µl x 2

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