Description

The CD8a/CD8b Jurkat Cell Line are human T lymphocyte Jurkat cells (Clone# E6-1) engineered to express CD8a (NM_ 001768.6) and CD8b (NM_004931.5) driven by an EF1a promoter. The cells were generated by transduction with CD8a/CD8b Lentivirus (BPS Bioscience #78650), which is a SIN (self-inactivating) lentivirus. The cells also express very low levels of endogenous CD4.

Background

CD8 (cluster of differentiation 8) is a cell surface glycoprotein found on most cytotoxic T lymphocytes that functions within the immune system to mediate cell-cell interactions. CD8 is a co-receptor for the TCR (T cell receptor) in T cells, binding to MHC (major histocompatibility complex) class I proteins. CD8 is a typical marker of cytotoxic T cells and is involved in signaling. CD8 has two isoforms, a and b. CD8b recruits Lck (lymphocyte-specific protein tyrosine kinase) to the TCR-CD3 complex, and Lck phosphorylates multiple proteins involved in activation of cytotoxic T lymphocytes. It is thus critical for the lysis of cancer cells. Studying CD8 and its importance in iso-immunity can further our understanding of post-transplant recognition and its potential for cancer therapy.

Application

Study CD8/MHCI complex formation and inhibition.

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

Jurkat (clone# E6-1), human T lymphocyte 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

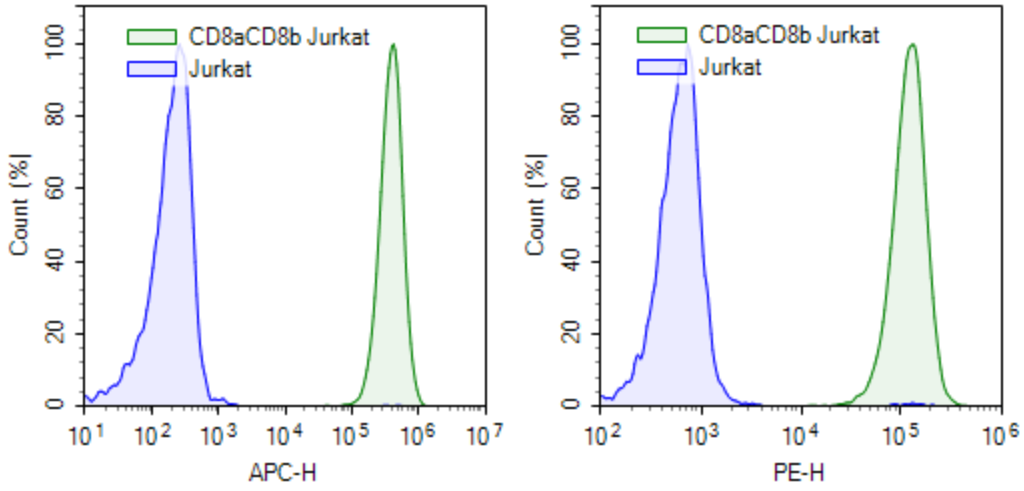

Figure 1. CD8a and CD8b expression in the CD8aCD8b Jurkat Cell Line.
The human CD8a/CD8b Jurkat cell line was stained using APC anti-human CD8 Antibody (Biolegend #344722; left panel) and PE anti-human CD8b Recombinant Antibody (Biolegend #376703; right panel), and the expression of CD8aCD8b was analyzed by flow cytometry.

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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.

Related Products

Products	*Catalog #*	*Size*
CD8aCD8b Lentivirus	78650	500 µl x 2
CD8a Lentivirus	78648	500 µl x 2
Anti-CD8 Antibody, Biotin-Labeled	101779	50 µg/100 µg