

Description

B2M Knockout Raji Cell Line is a Raji cell line where B2M (Beta-2-Microglobulin) has been genetically removed by CRISPR/Cas9 genome editing.

This cell line has been validated by sequencing and flow cytometry.

Background

Beta-2-Microglobulin is a required component of Major Histocompatibility Complex (MHC) class I molecules, which present peptide fragments from within the cell to cytotoxic T cells as part of the adaptive immune system. B2M plays an essential role both in governing MHC class I molecule stability and in promoting antigen binding and presenting the antigen to CD3/TCR complex of CD8⁺ T cells. Class I MHC molecules are present on the surface of all nucleated cells and play a role in the rejection of organs or allogenic cells during organ transplantation and cell therapy. B2M is an attractive target to reduce the immunogenicity of cell-derived allogenic cell therapies.

Application

- Study the impact of B2M knock-down.
- Study T cell activation, antigen presentation, and immune responses.

Materials Provided

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

Parental Cell Line

Raji human B lymphoblastoid cell line, derived from a patient with Burkitt lymphoma. Suspension 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 2	BPS Bioscience #60184

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% CO₂. 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 (ATCC modification), supplemented with 10% FBS, 1% Penicillin/Streptomycin

Cell Culture Protocol

Note: Note: Raji 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 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 and incubate at 37°C in a 5% CO₂ 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 10⁶ cells/ml. At first passage and subsequent passages, use Thaw Medium 2.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10⁶ cells/ml, but no less than 0.2 x 10⁶ cells/ml in Thaw Medium 2. The sub-cultivation ratio should maintain the cells between 0.2 x 10⁶ cells/ml and 2 x 10⁶ 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 10⁶ 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.

- 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.

A. Validation Data

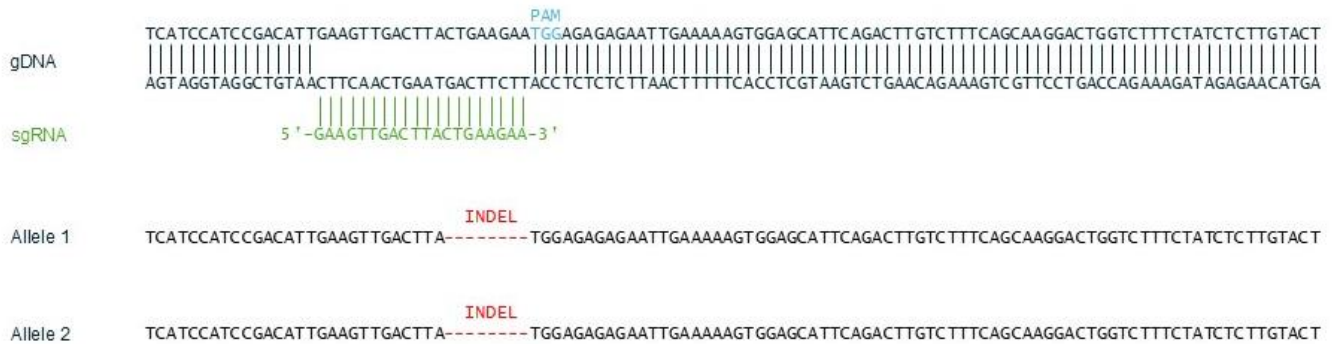


Figure 1. Genomic Sequencing of B2M in the B2M Knockout Raji Cell Line.

Genomic DNA from the B2M Knockout Raji cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green, and the Indels (Insertions/Deletions) in the two B2M alleles are indicated in red.

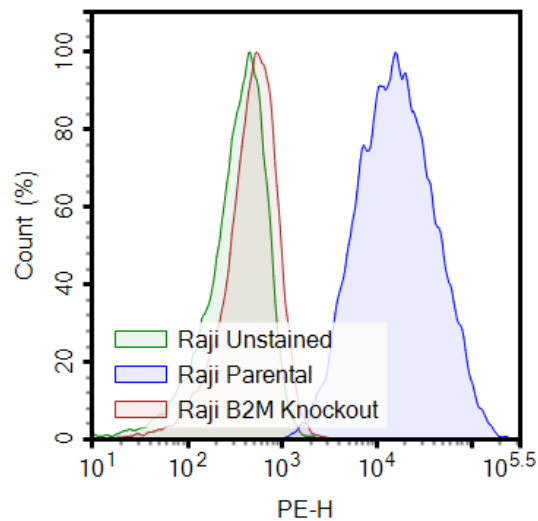


Figure 2. Expression of B2M in the B2M Knockout Raji Cell Line by flow cytometry.

B2M Knockout Raji cells (red) or parental Raji cells (blue) were stained with PE anti-human β 2-microglobulin Antibody (BioLegend #395703) and analyzed by flow cytometry. Unstained parental Raji cells are shown in green. Y-axis is the % cell number. X-axis is the intensity of PE.

Data shown is representative.

Sequence

Human mRNA for Beta-2-microglobulin (NCBI Reference Sequence #NM_004048.4), with the sgRNA targeting sequence underlined:

atgtctcgctccgtggccttagctgtgctcgcgctactctctcttctggcctggaggctatccagcgtactccaaagattcaggtttactcacgtcatcca
gcagagaatggaaagtcaaatttctgaattgctatgtgtctgggttcatccatccgacattgaagttgacttactgaagaatggagagagaattgaa
aaagtgagcattcagactgtcttccagcaaggactggtcttctatcttctgtactacactgaattcaccctcactgaaaaagatgagtatgcctgcc
gtgtgaacctgtgactttgtcacagccaagatagttaagtgggatcgagacatgtaa

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Troubleshooting Guide

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

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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
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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