

**Description**

B2M (Beta-2-Microglobulin) has been genetically removed by CRISPR/Cas9 genome editing from Jurkat cells.

**Background**

Beta-2-Microglobulin is a required component of Major Histocompatibility Complex (MHC) class 1 molecules, which present peptide fragments from within the cell to cytotoxic T cells as part of the adaptive immune system. The protein forms amyloid fibrils in some pathological conditions. A mutation in this gene drives hypercatabolic hypoproteinemia. 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.

**Applications**

- Study the consequences 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 $2 \times 10^6$ cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

**Parental Cell Line**

Jurkat (clone E6-1), human T lymphoblast, 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	<a href="#">BPS Bioscience #60184</a>

**Storage Conditions**

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a  $-80^{\circ}\text{C}$  freezer for long term storage.

Contact technical support at [support@bpsbioscience.com](mailto:support@bpsbioscience.com) if the cells are not frozen in dry ice upon arrival.

**Media Formulations**

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

**Media Required for Cell Culture**

*Thaw Medium 2 (BPS Bioscience #60184):*

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

**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 contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2.  
**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% CO<sub>2</sub> 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% CO<sub>2</sub> 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<sup>6</sup> 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 10<sup>6</sup> cells/ml, at no less than 0.2 x 10<sup>6</sup> cells/ml of Thaw Medium 2. The sub-cultivation ratio should maintain the cells between 0.2 x 10<sup>6</sup> cells/ml and 2 x 10<sup>6</sup> 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10<sup>6</sup> cells/ml.
2. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.



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

**Genomic Sequencing of B2M in the Jurkat cells**



*Figure 1: Genomic Sequencing of B2M in the B2M Knockout Jurkat Cell Line.*

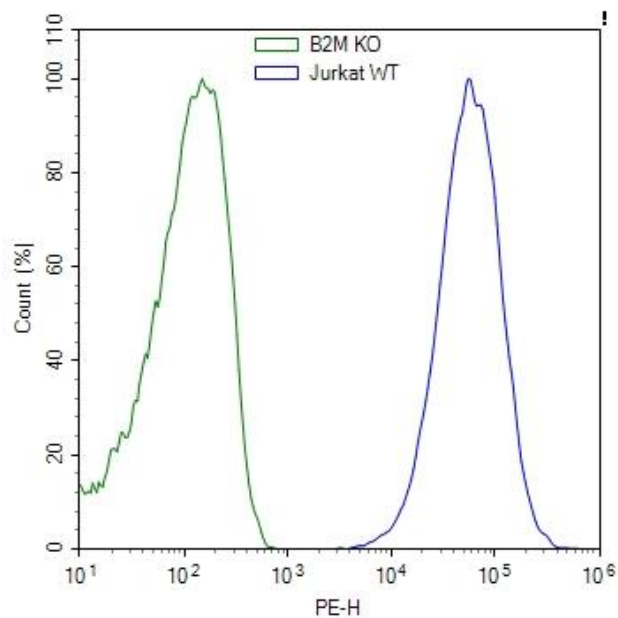
Genomic DNA from the B2M Knockout Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) and the Indels (Insertions / Deletions) in the two B2M alleles are labeled. The B2M genomic DNA is labeled as Ref.

**Sequence**

Human beta-2-microglobulin (B2M), mRNA, NCBI Reference Sequence: NM\_004048.4, with the sgRNA targeting sequence underlined:

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ATTCCTGAAGCTGACAGCATTCTGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCT
GGAGGCTATCCAGCGTACTCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTTCTGAATTGC
TATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAAATGGAGAGAGAATTGAAAAAGTGGAGCATTCA
GACTTGTCTTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCCACTGAAAAAGATGAGTATGCCTG
CCGTGTGAACCATGTGACTTTGTACAGCCCAAGATAGTTAAGTGGGATCGAGACATGTAAGCAGCATCATGGAGGTTTGA
AGATGCCGCATTTGGATTGGATGAATTCCAAATTCTGCTTGCTTGCTTTTTAATATTGATATGCTTATACACTTACACTTTATG
CACAAAATGTAGGGTTATAATAATGTTAACATGGACATGATCTTCTTTATAATTCTACTTTGAGTGCTGTCTCCATGTTTGTG
GTATCTGAGCAGGTTGCTCCACAGGTAGCTCTAGGAGGGCTGGCAACTTAGAGGTGGGGAGCAGAGAATTCTCTTATCCAA
CATCAACATCTTGGTCAGATTTGAACTCTTCAATCTCTTGCACTCAAAGCTTGTTAAGATAGTTAAGCGTGCATAAGTTAACT
TCCAATTTACATACTCTGCTTAGAATTTGGGGGAAAAATTTAGAAATATAATTGACAGGATTATTGAAAATTTGTTATAATGAA
TGAAACATTTTGTATATAAGATTCATATTTACTTCTTATACATTTGATAAAGTAAGGCATGGTTGTGGTTAATCTGGTTTATT
TTTGTCCACAAGTTAAATAAATCATAAACTTGA
  
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**FACS Analysis of B2M Expression**

*Figure 2: B2M Expression in B2M knockout Jurkat cells.*

Flow cytometry was performed using a PE-labeled anti-human B2M antibody (BioLegend #395703). Parental Jurkat cells (Blue) were compared to B2M Knockout Jurkat cells (Green). The Y-axis is the % cell number. The X-axis is the intensity of PE.

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**Troubleshooting Guide**

Visit [bpsbioscience.com/cell-line-faq](https://bpsbioscience.com/cell-line-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
TCR/B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line	78364	2 vials
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