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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Both B2M (Beta-2-Microglobulin) and CIITA (Class II Transactivator) have been genetically removed from THP-1 cells using CRISPR/Cas9 genome editing.

Background

Beta-2-Microglobulin is a required component of Major Histocompatibility Complex (MHC) class I molecules, which present peptide fragments from within a 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.

CIITA (Class II Transactivator, also known as Class II Major Histocompatibility Complex Transactivator) acts as a coactivator for MHC class II-specific gene expression. IFN-Y induces CIITA gene expression via the JAK1 and STAT1 pathways. Defects in CIITA have been implicated in Bare Lymphocyte Syndrome (BLS), which is characterized by the absence of MHC class II transcription and severe immunodeficiencies.

Application

1. Study the consequences of deficient MHC class I and II.
2. Study T cell activation, antigen presentation, and immune responses.
3. Useful for the development of improved universal CAR-T or other effector cells.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience, #79796)

Parental Cell Line

THP-1, human monocyte, suspension, derived from an acute monocytic leukemia patient.

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, 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.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 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

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_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% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged in Thaw Medium 2 before they reach a density of 2 x 10^6 cells/ml.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Thaw Medium 2. 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 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. The next day, transfer the vials to liquid nitrogen for 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/CIITA Double Knockout THP-1 Cell Line. Genomic DNA from the B2M and CIITA Double Knockout THP-1 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two B2M alleles are highlighted in red. The Cas9 cut site is indicated with the black vertical dotted line. The parental B2M genomic DNA is labeled as Ref.

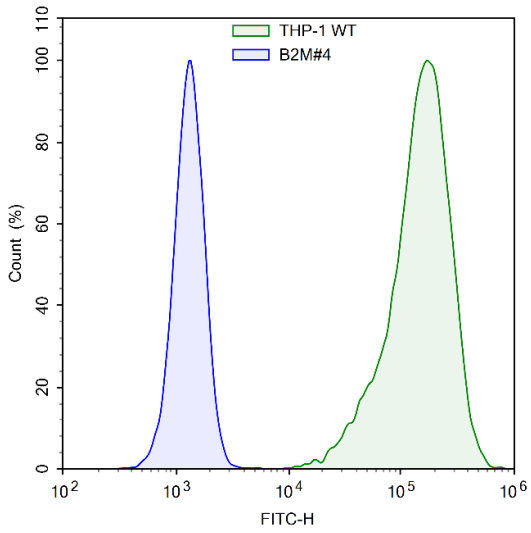

Figure 2: B2M Expression in B2M/CIITA Double Knockout THP-1 cells. Flow cytometry was performed using anti-human HLA-ABC Alexa Fluor 488 antibody (BD Biosciences, #560169). Parental THP-1 cells (green) were compared to B2M/CIITA Double Knockout THP-1 cells (blue). The Y-axis is the % cell number. The X-axis is the intensity of Alexa Fluor.

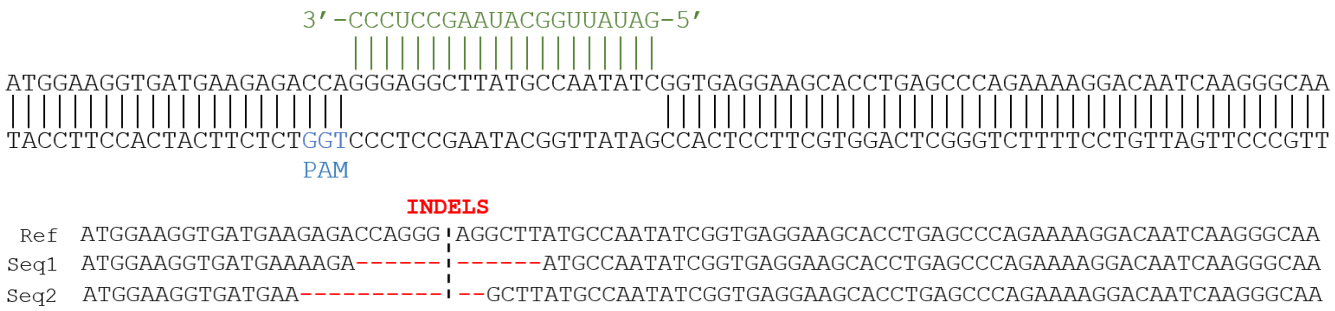

Figure 3: Genomic Sequencing of CIITA in the B2M and CIITA Double Knockout THP-1 Cell Line. Genomic DNA from the B2M/CIITA Double Knockout THP-1 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two CIITA alleles are highlighted in red. The Cas9 cut site is indicated with the black vertical dotted line. The parental CIITA genomic DNA is labeled as Ref.

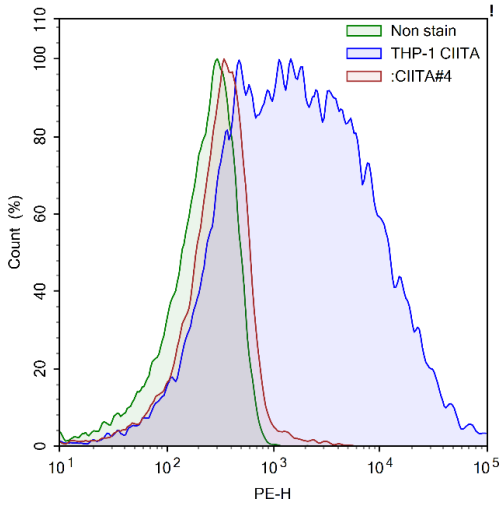

Figure 4: CIITA Expression in B2M/CIITA Double Knockout THP-1 cells. Flow cytometry was performed using a PE-labeled anti-human HLA-DR antibody (R&D Systems, #FAB4869P-100). Unstained parental THP-1 cells (green) and stained parental THP-1 cells (blue) were compared to B2M/CIITA Double Knockout THP-1 cells (red). The Y-axis is the % cell number. The X-axis is the intensity of PE.

Sequences

Human Beta-2-Microglobulin (B2M), mRNA, NCBI Reference Sequence: NM_004048.4, with the sgRNA targeting sequence underlined:

ATTCCTGAAGCTGACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCT
GGAGGCTATCCAGCGTACTCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTTCCTGAATTGC
TATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATTCA
GACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCCACTGAAAAAGATGAGTATGCCTG
CCGTGTGAACCATGTGACTTTGTCACAGCCCAAGATAGTTAAGTGGGATCGAGACATGTAAGCAGCATCATGGAGGTTTGA
AGATGCCGCATTTGGATTGGATGAATTCCAAATTCTGCTTGCTTGCTTTTTAATATTGATATGCTTATACACTTACACTTTATG
CACAAAATGTAGGGTTATAATAATGTTAACATGGACATGATCTTCTTTATAATTCTACTTTGAGTGCTGTCTCCATGTTTGAT
GTATCTGAGCAGGTTGCTCCACAGGTAGCTCTAGGAGGGCTGGCAACTTAGAGGTGGGGAGCAGAGAATTCTCTTATCCAA
CATCAACATCTTGGTCAGATTTGAACTCTTCAATCTCTTGCACTCAAAGCTTGTTAAGATAGTTAAGCGTGCATAAGTTAACT
TCCAATTTACATACTCTGCTTAGAATTTGGGGGAAAATTTAGAAATATAATTGACAGGATTATTGGAAATTTGTTATAATGAA
TGAAACATTTTGTCATATAAGATTCATATTTACTTCTTATACATTTGATAAAGTAAGGCATGGTTGTGGTTAATCTGGTTTATT
TTTGTTCCACAAGTTAAATAAATCATAAAACTTGA

Human Class II Major Histocompatibility Complex Transactivator (CIITA), RefSeqGene (LRG_49) on chromosome 16. NCBI Reference Sequence: NM_000246.4, with the sgRNA targeting sequence underlined:

ATGCGTTGCCTGGCTCCACGCCCTGCTGGGTCCTACCTGTCAGAGCCCCAAGGCAGCTCACAGTGTGCCACCATGGAGTTG
GGGCCCCTAGAAGGTGGCTACCTGGAGCTTCTTAACAGCGATGCTGACCCCCTGTGCCTCTACCACTTCTATGACCAGATGG
ACCTGGCTGGAGAAGAAGAGATTGAGCTCTACTCAGAACCCGACACAGACACCATCAACTGCGACCAGTTCAGCAGGCTGT
TGTGTGACATGGAAGGTGATGAAGAGACCAGGGAGGCTTATGCCAATATCGCGGAACTGGACCAGTATGTCTTCCAGGAC
TCCCAGCTGGAGGGCCTGAGCAAGGACATTTTCAAGCACATAGGACCAGATGAAGTGATCGGTGAGAGTATGGAGATGCC
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GCTGTTCAACCAGGAGCCAGCCTCCGGCCAGATGCGCCTGGAGAAAACCGACCAGATTCCCATGCCTTTCTCCAGTTCCTCG
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TTGCACCCTCCTCCTCACAGCCCGGCCCCGGGGCCGCCTGGTCCAGAGCCTGAGCAAGGCCGACGCCCTATTTGAGCTGTCC
GGCTTCTCCATGGAGCAGGCCCAGGCATACGTGATGCGCTACTTTGAGAGCTCAGGGATGACAGAGCACCAAGACAGAGC
CCTGACGCTCCTCCGGGACCGGCCACTTCTTCTCAGTCACAGCCACAGCCCTACTTTGTGCCGGGCAGTGTGCCAGCTCTCA
GAGGCCCTGCTGGAGCTTGGGGAGGACGCCAAGCTGCCCTCCACGCTCACGGGACTCTATGTCGGCCTGCTGGGCCGTGC
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TACAGGAGGACCAGTTCCCATCCGCAGACGTGAGGACCTGGGCGATGGCCAAAGGCTTAGTCCAACACCCACCGCGGGCC

GCAGAGTCCGAGCTGGCCTTCCCCAGCTTCCTCCTGCAATGCTTCCTGGGGGCCCTGTGGCTGGCTCTGAGTGGCGAAATCA
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CGGAAGACACAGCTGGGGAGCTCCCTGCTGTTCGGGACCTAAAGAAACTGGAGTTTGCGCTGGGCCCTGTCTCAGGCCCCC
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GATCGGGGACGAGGGTGTCTCGCAGCTCTCAGCCACCTTCCCCCAGCTGAAGTCCTTGGAAACCCTCAATCTGTCCCAGAAC
AACATCACTGACCTGGGTGCCTACAAACTCGCCGAGGCCCTGCCTTCGCTCGCTGCATCCCTGCTCAGGCTAAGCTTGTACA
ATAACTGCATCTGCGACGTGGGAGCCGAGAGCTTGGCTCGTGTGCTTCCGGACATGGTGTCCCTCCGGGTGATGGACGTCC
AGTACAACAAGTTCACGGCTGCCGGGGCCCAGCAGCTCGCTGCCAGCCTTCGGAGGTGTCCTCATGTGGAGACGCTGGCG
ATGTGGACGCCCACCATCCCATTCAGTGTCCAGGAACACCTGCAACAACAGGATTCACGGATCAGCCTGAGATGA

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.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
Firefly Luciferase THP-1 cell line	78409	2 vials
B2M Knockout THP-1 Cell Line	78389	2 vials
TCR/B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line	78364	2 vials
NFAT Reporter (Luciferase) THP-1 Cell Line	78320	2 vials
NF-κB Reporter (Luc) - THP-1 Cell Line	79645	2 vials
CIITA Knockout THP-1 Cell Line	78390	2 vials
B2M Knockout Jurkat Cell Line	78342	2 vials