Description

CIITA Knockout THP-1 Cell Line is a THP-1 cell line where CIITA (Class II Transactivator) has been genetically removed from THP-1 cells using CRISPR/Cas9 genome editing.

Background

CIITA (Class II Transactivator, also known as Class II Major Histocompatibility Complex Transactivator) acts as a coactivator for MHC class II-specific gene expression, and negatively regulates IL-4 expression during T cell differentiation. IFN-Y induces CIITA gene expression via the JAK1 and STAT1 pathways. The GTP-binding and acidic, proline-serine-threonine rich regions of CIITA appear to be required for its activity. 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

- Study the effects of MHC class II loss.
- Study T cell activation, antigen presentation, and immune responses.
- Development of improved universal CAR-T or other effector cells.

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

THP-1, human monocyte, 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

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% 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 and 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 2 to the conical tube containing the cells. Thaw Medium 2 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for viability. For a T25 flask, add 3-4 ml of fresh Thaw Medium 2 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they reach 2 x 10^6 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 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, in 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 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. The next day, transfer the vials to liquid nitrogen 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

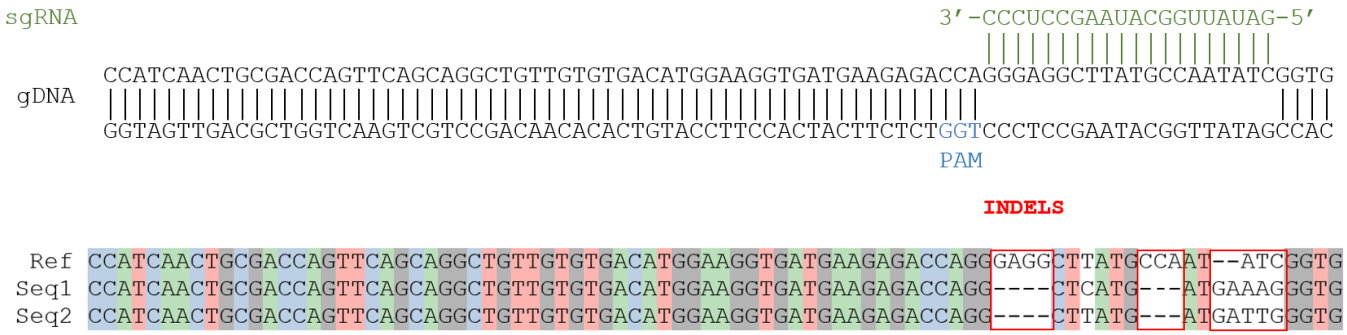

Figure 1: Genomic Sequencing of CIITA in the CIITA Knockout THP-1 Cell Line.
Genomic DNA from the CIITA Knockout THP-1 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two CIITA alleles are highlighted in red. The CIITA genomic DNA is labeled as Ref.

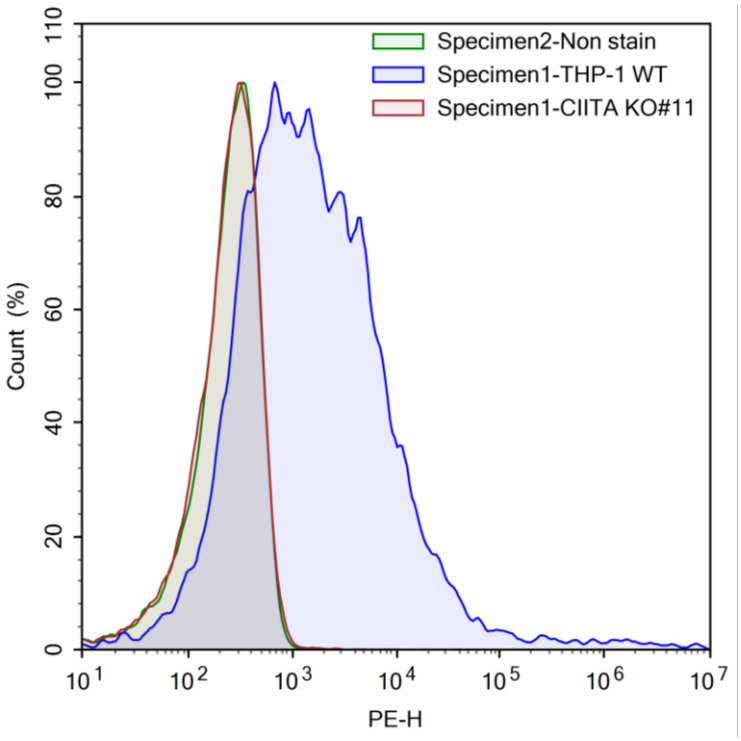

Figure 2: CIITA Expression in CIITA knockout THP-1 Cell Line.
Cells were stained with Human HLA-DR PE-conjugated Antibody (R&D Systems #FAB4869P-100) and analyzed by flow cytometry. Unstained parental THP-1 cells (green) and stained parental THP-1 cells (blue) were compared to CIITA Knockout THP-1 cells (red). The Y-axis represents % cell number. The X-axis indicates the PE intensity.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence

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
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TTGCACCCTCCTCCTCACAGCCCGGCCCCGGGGCCGCCTGGTCCAGAGCCTGAGCAAGGCCGACGCCCTATTTGAGCTGTCC
GGCTTCTCCATGGAGCAGGCCCAGGCATACGTGATGCGCTACTTTGAGAGCTCAGGGATGACAGAGCACCAAGACAGAGC
CCTGACGCTCCTCCGGGACCGGCCACTTCTTCTCAGTCACAGCCACAGCCCTACTTTGTGCCGGGCAGTGTGCCAGCTCTCA
GAGGCCCTGCTGGAGCTTGGGGAGGACGCCAAGCTGCCCTCCACGCTCACGGGACTCTATGTCGGCCTGCTGGGCCGTGC
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TACAGGAGGACCAGTTCCCATCCGCAGACGTGAGGACCTGGGCGATGGCCAAAGGCTTAGTCCAACACCCACCGCGGGCC
GCAGAGTCCGAGCTGGCCTTCCCCAGCTTCCTCCTGCAATGCTTCCTGGGGGCCCTGTGGCTGGCTCTGAGTGGCGAAATCA
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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
B2M/CIITA Double Knockout THP-1 Cell Line	78391	2 vials
B2M Knockout Jurkat Cell Line	78342	2 vials