

**Description**

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- $\gamma$  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**

1. Study the consequences of losing MHC class 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 $2 \times 10^6$ 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	<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.



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<sub>2</sub>. 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 and 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 in Thaw medium 2 before they reach a density of 2 x 10<sup>6</sup> cells/ml.

#### *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.

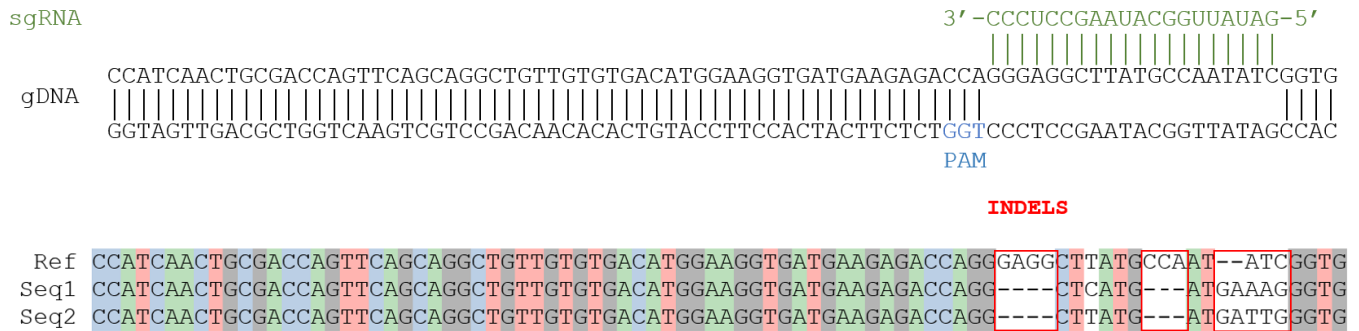
#### *Cell Freezing*

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. The next day, transfer the vials to liquid nitrogen for storage.

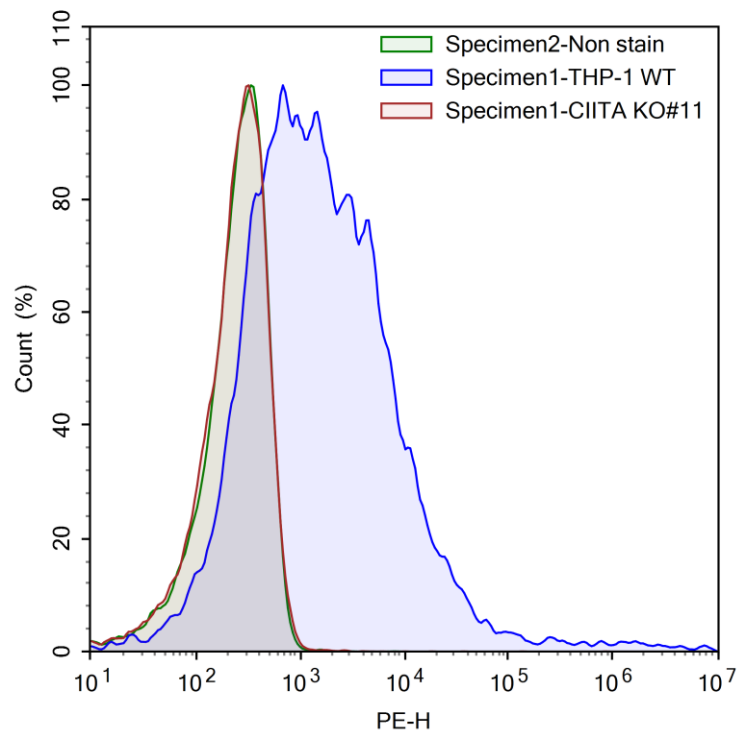


Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

**A. Validation Data**



**Figure 1: Genomic Sequencing of 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 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 CIITA Knockout THP-1 cells (red). The Y-axis is the % cell number. The X-axis is the intensity of PE.

**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  
 ACCTGGCTGGAGAAGAAGAGATTGAGCTCTACTCAGAACCCGACACAGACACCATCAACTGCGACCAGTTTCAGCAGGCTGT  
 TGTGTGACATGGAAGGTGATGAAGAGACCGGGAGGCTTATGCCAATATCGCGGAACTGGACCAGTATGTCTTCCAGGAC  
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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**

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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>
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- $\kappa$ B 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