

**Description**

CD36 Knockout THP-1 Cell Line is a THP-1 cell line where CD36 (Cluster of Differentiation 36) has been genetically removed from THP-1 cells using CRISPR/Cas9 genome editing via the use of a lentivirus encoding CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human CD36.

**Background**

CD36 is a membrane glycoprotein present on platelets, mononuclear phagocytes, adipocytes, hepatocytes, myocytes, and some epithelia. On microvascular endothelial cells, CD36 is a receptor for thrombospondin-1 and related proteins and functions as a negative regulator of angiogenesis. On phagocytes, through its functions as a scavenger receptor recognizing specific oxidized phospholipids and lipoproteins, CD36 participates in internalization of apoptotic cells, certain bacterial and fungal pathogens, and modified low-density lipoproteins, thus contributing to inflammatory responses and atherothrombotic diseases. CD36 also binds long-chain fatty acids and facilitates their transport into cells, thus participating in muscle lipid utilization, adipose energy storage, and gut fat absorption and possibly contributing to the pathogenesis of metabolic disorders, such as diabetes and obesity. CD36 in many cells is localized in specialized cholesterol-rich membrane microdomains and may also interact with other membrane receptors, such as tetraspanins and integrins. Identification of the precise CD36 signaling pathways in specific cells elicited in response to specific ligands may yield novel targets for drug development.

**Application**

- Study cellular mechanisms and diseases related to CD36 knockout.
- Introduce further CRISPR/Cas9-based genetic manipulations in order to understand the interplay between CD36 and other partners and pathways.

**Materials Provided**

Components	Format
2 vials of frozen cells	Each vial contains $>1 \times 10^6$ 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>
Growth Medium 2M	<a href="#">BPS Bioscience #78181</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°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. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO<sub>2</sub>. 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.

*Growth Medium 2M (BPS Bioscience #78181):*

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 µg/ml of Puromycin.

**Cell Culture Protocol**

**Note: THP-1 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% 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 Growth Medium 2M.

*Cell Passage*

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10<sup>6</sup> cells/ml, but no less than 0.2 x 10<sup>6</sup> cells/ml, with Growth Medium 2M. 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 Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10<sup>6</sup> 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. 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

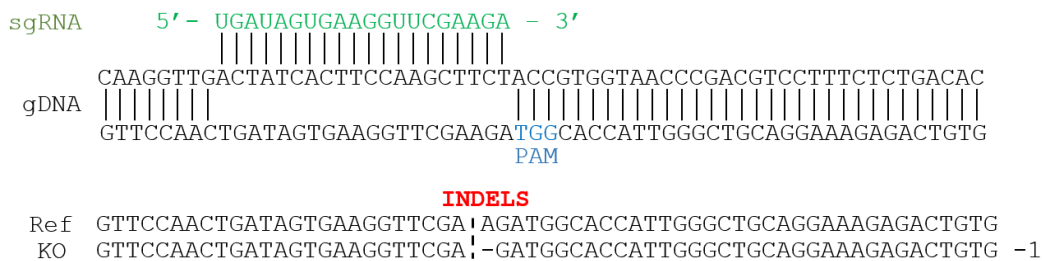


Figure 1: Genomic sequencing of CD36 in the CD36 Knockout THP-1 Cell Line.

Genomic DNA from the CD36 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 CD36 alleles are labeled. The CD36 genomic DNA is labeled as reference.

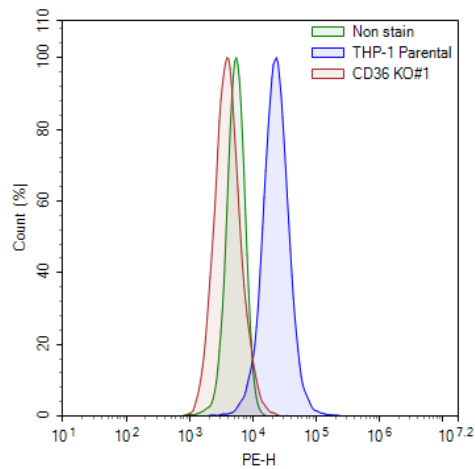


Figure 2: CD36 expression in CD36 Knockout THP-1 cells.

Parental THP-1 cells (blue) and CD36 Knockout THP-1 cells (green) were stained with PE anti-human CD36 Antibody (BioLegend #336205) and analyzed by flow cytometry. Unstained THP-1 cells were used as negative control (non-specific staining, red). The Y-axis represents the % cell number. The X-axis indicates the intensity of PE.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

### Sequence

Human CD36 mRNA, NCBI Reference Sequence: NM\_001371077, with the sgRNA targeting sequence (Antisense) underlined:

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### 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).

### 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

Products	Catalog #	Size
CD36 HEK293 Cell Line (High or Medium Expression)	78526	2 vials
CD36, Avi-His-Tag Recombinant	101184	100 µg
CD36, FC Fusion, Avi-Tag Recombinant	101813	25 µg/100 µg

Version 110623