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Produktinformation

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

FcGR1a (CD64) Knockout THP-1 Cell Line is a THP-1 cell line in which FcGR1a (Fc Gamma Receptor Ia or CD64) has been genetically removed from THP-1 cells using CRISPR/Cas9 genome editing with a lentivirus encoding CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human FcGR1a (CD64).

Background

FcGR1a (Fc Gamma Receptor Ia), also known as CD64, is a 72 kDa transmembrane glycoprotein that with CD32 and CD16 receptors form the large immunoglobulin superfamily. This protein is a high-affinity Fc-gamma receptor and plays an important role in both innate and adaptive immune responses. It can be found in monocytes, macrophages, and dendritic cells (DCs), but is absent in neutrophils under normal conditions. During infection the expression of FcGR1 is quickly increased in neutrophils, clustering in the cell membrane and allowing rapid binding and internalization of immune complexes. It mediates IgG effector functions in macrophages, triggering antibody-dependent cellular cytotoxicity (ADCC) of virus-infected cells and clearance of immune complexes. Diseases associated with FcGR1a include peritonitis and pharyngitis, but it is also linked to AML (acute monocytic leukemia) subtypes M4 and M5. Among its related pathways are ADORA2B (adenosine A2B receptor)-mediated anti-inflammatory cytokine production and regulation of actin dynamics for phagocytic cup formation. FcGR1a has been studied in the treatment of diseases related to macrophage-mediated chronic inflammation, such as rheumatoid arthritis (RA) and chronic diabetic wounds (CDW), and AML. Recently, fusion proteins made of CD64/CD16A have been used to create armed iPSC derived NK cells, resulting in sustained and robust ADCC in a mouse model. The use of FcGR1a-directed immunotoxins or cytolytic fusion proteins may open new avenues of treatment in multiple diseases linked to this protein.

Application

- Study phenotype changes related to FcGR1a (CD64) knockout.
- Introduce further CRISPR/Cas9-based genetic manipulations to understand the interplay between FcGR1a (CD64) and other partners and pathways.
- Use as negative control in experiments involving CD64 related ADCC.

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
Growth Medium 2M	BPS Bioscience #78181

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_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, 1% Penicillin/Streptomycin.

Growth Medium 2M (BPS Bioscience #78181):

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 µg/ml of Hygromycin B.

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_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 before they reach a density of 2 x 10^6 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 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml of Growth Medium 2M. 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. 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.

Validation Data

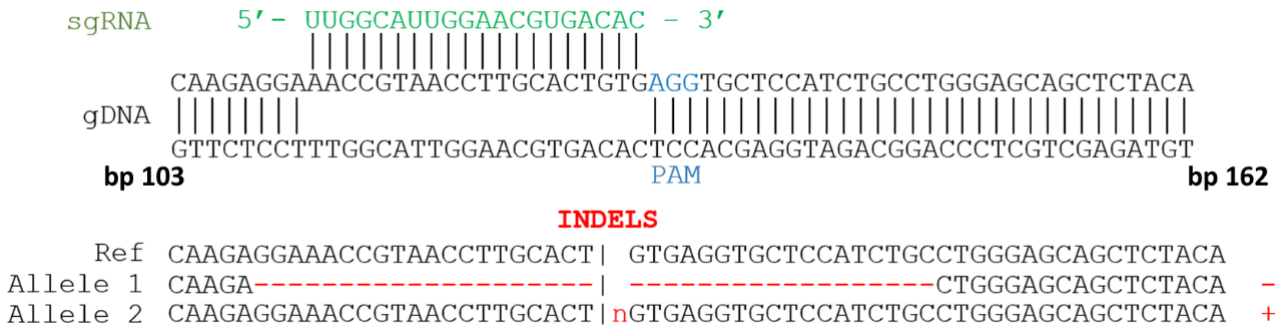

Figure 1: Genomic sequencing of FcGR1a (CD64) in the FcGR1a (CD64) Knockout THP-1 Cell Line. Genomic DNA from the FcGR1a (CD64) Knockout THP-1 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green and the Indels (Insertions/Deletions) in the FcGR1a (CD64) alleles are labeled in red. The FcGR1a (CD64) genomic DNA is labeled as reference.

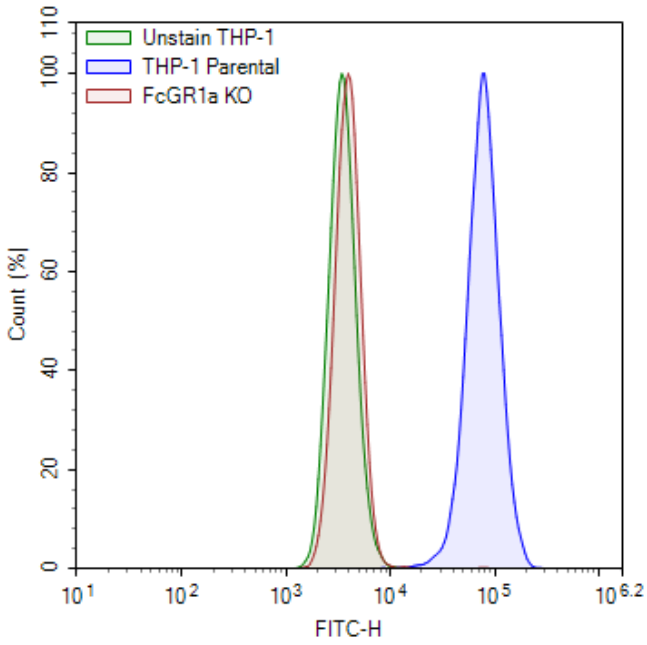

Figure 2: Expression of FcGR1a (CD64) expression in FcGR1a (CD64) Knockout THP-1 Cell Line by flow cytometry.
Parental THP-1 cells (blue) and FcGR1a (CD64) Knockout THP-1 cells (green) were stained with FITC-conjugated anti-human CD64 Antibody (BioLegend #399505) and analyzed by flow cytometry. Unstained 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 FITC.

Data are representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

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.

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.

References
Xu J. and Guo Y., 2020 *Front Mol Biosci* 7:581615.
Akinrinmade O., *et al.*, 2017 *Biomedicines* 5(3): 56.

Related Products

Products	*Catalog #*	*Size*
Adenosine A2A Receptor Functional HEK293 Cell Line	79381	2 vials
Firefly Luciferase THP-1 Cell Line	78409	2 vials
NFAT Luciferase Reporter THP-1 Cell Line	78320	2 vials
Cas9 Lentivirus (Puromycin Selection)	78066	500 µl x 2
AAV-DJ SaCas9	78478	50 µl x 2

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