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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic)



Description

The FCGR2A Knockout Jurkat cell line was generated by CRISPR/Cas9 genome editing to remove FCGR2A (CD32A), the gene encoding protein FcγRIIa (Fragment crystallizable gamma receptor II a, also known as FcγRIIa, Fc-gamma-RIIa, and CD32A).

Background

FCGR2A (also known as CD32A) encodes a low affinity Fc receptor for immunoglobulin G. FcγRIIa is a cell surface receptor expressed on a variety of immune cells such as macrophages and neutrophils. It is involved in phagocytosis and in the clearing of spent immune complexes from the circulation. A polymorphism in FcγRIIa is associated with increased risks of nephritis and lupus.

Application(s)

Use as a control for characterizing FCGR2A in T cells

Materials Provided

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

Parental Cell Line

Jurkat (clone E6-1), human T lymphoblast, 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, 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. Cells should be grown at 37°C with 5% CO₂. 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% CO₂ 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% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they reach a density of 2×10^6 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 2×10^6 cells/ml, at no less than 0.2×10^6 cells/ml of Thaw Medium 2. The sub-cultivation ratio should maintain the cells between 0.2×10^6 cells/ml and 2×10^6 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 $\sim 2 \times 10^6$ 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. Transfer the vials to liquid nitrogen the next day 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

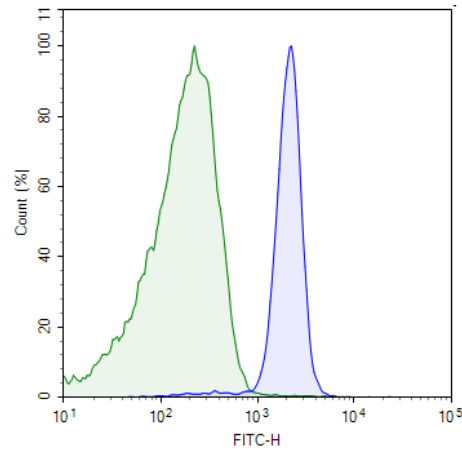


Figure 2: Expression of FCGR2A (CD32A) in the FCGR2A (CD32A) Knockout Jurkat Cell Line. Parental Jurkat cells (blue) or FCGR2A (CD32A) Knockout Jurkat cells (green) were stained with CoraLite 488-conjugated FCGR2A antibody (ThermoFisher #CL488-66529) and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of FITC.

Sequence

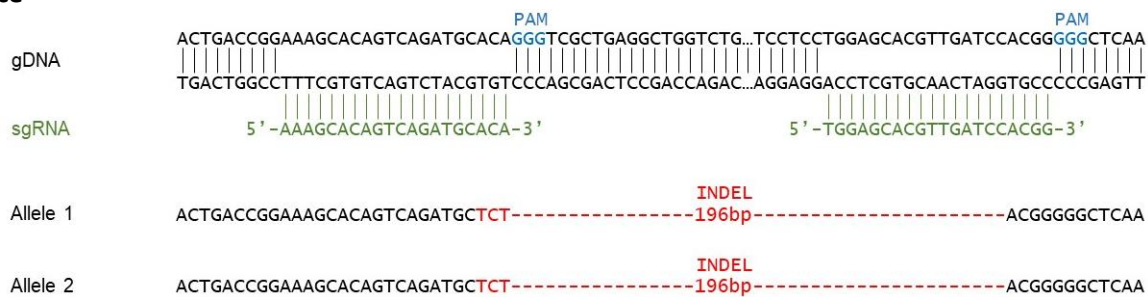


Figure 1. Genomic Sequencing of FCGR2A (CD32A) in the FCGR2A (CD32A) Knockout Jurkat cells. Genomic DNA from the FCGR2A (CD32A) Knockout Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) sites are shown in blue, the sgRNA (synthetic guide RNA) sequences in green, and the Indels (Insertions / Deletions) in the two FCGR2A alleles are indicated in red.

License Disclosure

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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
FCGR2A CRISPR/Cas9 Lentivirus (Integrating)	78537	500 µl x 2
Fc (IgG1): FcRn Inhibitor Screening Colorimetric Assay Kit	78501	96 reactions
TCR Activator/FcGR2B CHO Cell Line	78436	2 vials
FcGR3A (CD16A) CHO Cell Line	78332	2 vials