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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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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

CD20 Knockout Raji Cell Line is a Raji cell line where CD20 (B-lymphocyte antigen CD20, or MS4A1) has been genetically removed from Raji cells using CRISPR/Cas9 genome editing.

This cell line has been validated by genome sequencing and flow cytometry.

Background

The Raji line was established from a Burkitt's lymphoma patient. Raji cells constitutively express B cell antigens CD19, CD20, and CD22, and offer a physiologically relevant platform to evaluate cancer-directed immunotherapies such as Chimeric Antigen Receptor (CAR) T-cells.

CD20 (also known as B-lymphocyte antigen CD20, or MS4A1) is a non-glycosylated protein expressed on the surface of B-cells during all stages of B-cell development following the pre-B phase. The function and natural ligand of CD20 is not entirely clear. However, CD20 is a marker for several B-cell malignancies, including B-cell lymphomas, B-cell chronic lymphocytic leukemia, and melanoma cancer stem cells. Accordingly, several anti-CD20 monoclonal antibodies have been developed to effectively deplete B-cell populations and manage B-cell malignancies, as well as some inflammatory and autoimmune diseases. The first anti-CD20 monoclonal antibody was Rituximab, which was approved by the FDA in 1997. More recently, anti-CD20-CD19 bispecific CAR-T cells have been developed to address concerns over potential relapse in cancer patients.

Application

Use as a control in CAR-T or NK co-culture killing assays.

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)

Host Cell

Raji human B lymphoblastoid cell line, derived from a patient with Burkitt lymphoma. Suspension cells.

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 this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results.

Materials Required for Cell Line Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184

Storage Conditions

Cells will arrive upon 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. Cells should be grown at 37°C with 5% CO₂. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Line Culture

Thaw Medium 2 (BPS Bioscience #60184):

RPMI-1640 medium (ATCC modification) supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: Raji 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% 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 x 10⁶. At first passage and subsequent passages, use Thaw Medium 2.

Cell Passage

Dilute the cell suspension into new culture vessels at a minimum of 0.2 x 10⁶ cells/ml in Thaw Medium 2. The recommended sub-cultivation ratio is 1:6 to 1:8 once or twice per week, so cells are maintained between 0.2 x 10⁶ cells/ml and 2 x 10⁶ 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 10⁶ 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

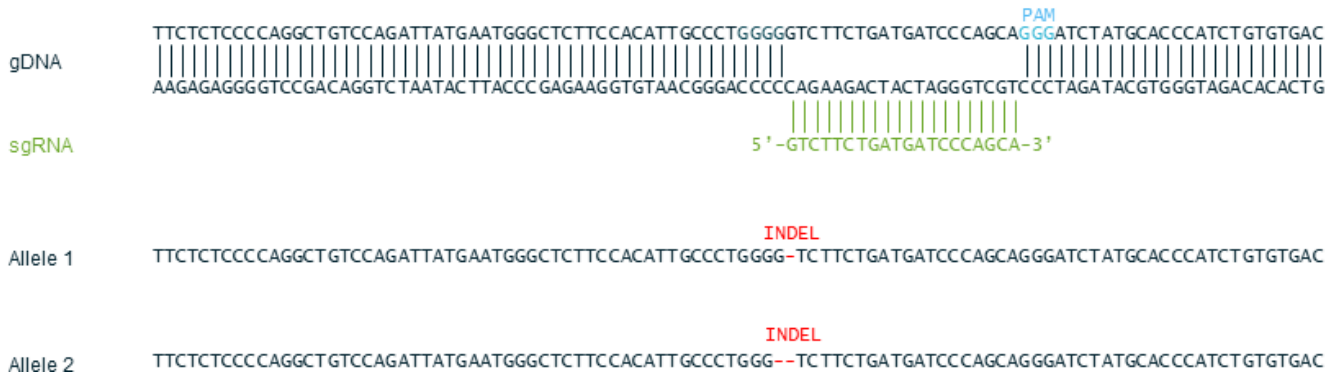


Figure 1. Genomic sequencing of CD20 in the CD20 Knockout Raji Cell Line.

Genomic DNA from the CD20 Knockout Raji 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 CD20 alleles are highlighted in red. The CD20 genomic DNA is labeled as gDNA.

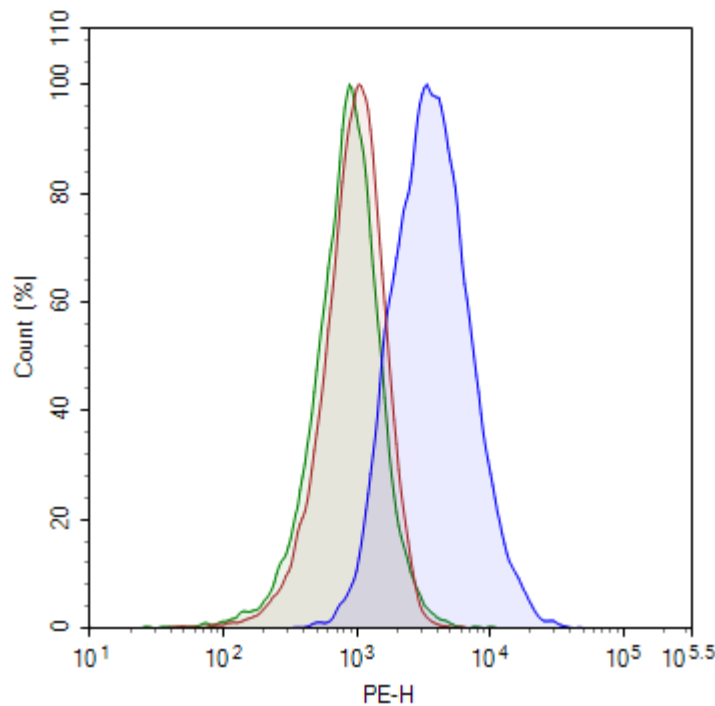


Figure 2. Flow cytometry analysis of CD20 expression in CD20 Knockout Raji Cell Line.

Cells were stained with PE Anti-CD20 antibody [2H7] (Abcam #AB112461) and analyzed by flow cytometry. The parental Raji cells are shown in blue, unstained parental Raji cells are shown in red, and the CD20 Knockout Raji cells are shown in green. The y axis shows the % of cells, while the x axis represents the fluorophore intensity.

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

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Anti-CD20 CAR-T Cells	78611	1 vial
Anti- CD20 CAR Lentivirus (Clone Leu-16 ScFv-CD8-4-1BB-CD3ζ)	78606	50 µl
Anti-CD20 Functional Antibody	71209	100 µg
Firefly Luciferase CD19 Knockout Raji Cell Line	82167	2 vials
Cas9 Expressing Raji Cell Line	78156	2 vials
CD20/ Firefly Luciferase CHO Cell Line	78620	2 vials

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