Description

Firefly Luciferase CD22 Knockout Raji Cell Line is a Raji cell line in which CD22 (Cluster of Differentiation 22, or SIGLEC2) is not expressed due to CRISPR/Cas9 genome editing. These cells also constitutively express firefly (*Photinus pyralis*) luciferase under the control of an EF1a promoter. This cell line was generated by transduction of the CD22 Knockout Raji Cell Line (BPS Bioscience #82970), which was created by electroporation with Cas9-sgRNA ribonucleoprotein complexes containing sgRNAs, with Firefly Luciferase Lentivirus (EF1a Promoter, Puromycin) (BPS Bioscience #78740-P).

This cell line has been validated by genome sequencing, flow cytometry, and luciferase activity measurement.

Background

The Raji cell 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 or CAR-NK cells.

CD22 (also known as Siglec-2) is expressed on the membrane of B cells. CD22 regulates the B cell immune response by acting as an inhibitory co-receptor of the B cell receptor (BCR), shown to influence BCR signaling as well as B cell survival and migration. CD22 is expressed on the cell surface of most B cell malignancies and can serve as a B cell-specific target for cancer immunotherapies. One area of interest is in developing CD22-targeting antibody-drug conjugates, as CD22 is frequently endocytosed from the cell membrane. In 2017, the FDA approved Besponsa (inotuzumab ozogamicin), an antibody-drug conjugate targeting CD22, for patients with B cell acute lymphoblastic leukemia (B-ALL). Additional therapies targeting CD22 are under evaluation. Identifying additional CD22-targeting therapies will be important for first-line treatment of B cell malignancies as well as treatment for patients who experience relapse due to antigen escape under therapies targeting other B cell markers.

Firefly luciferase has been used as a sensitive reporter to study a wide range of biological responses. The signal generated by the firefly luciferase reporter is proportional to Raji cell numbers and facilitates the quantification of Raji cells killing upon co-culture with CAR-T or NK cells.

Application

- Use as control in CAR-T or NK co-culture killing assays.
- *In vitro* and *in vivo* bioluminescence imaging.

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

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 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, 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: 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 (ATCC modification) medium supplemented with 10% FBS, 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% 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. 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 10^6 cells/ml in Thaw Medium 2. The recommended sub-cultivation ratio is 1:6 to 1:8 twice per week, so cells are maintained 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.

A. Validation Data

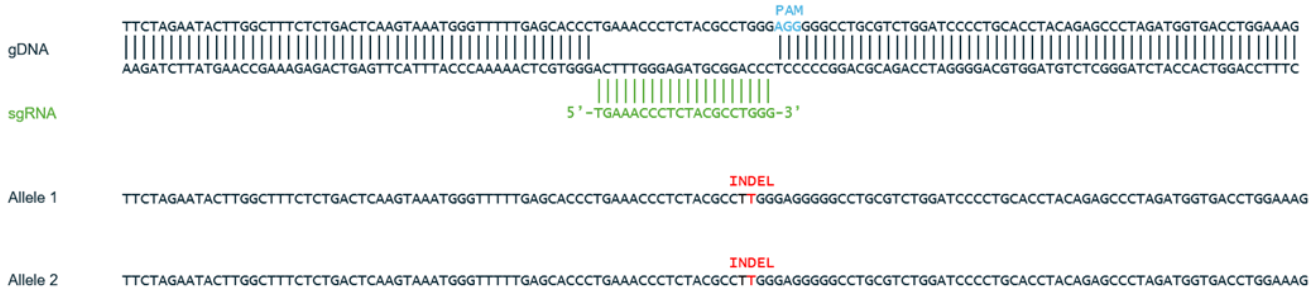

Figure 1. Genomic sequencing of CD22, exon 3, in the CD22 Knockout Raji Cell Line.
Genomic DNA from CD22 Knockout Raji cells (#82970) was isolated and sequenced. The CD22 Knockout Raji Cell Line was used as the parental cell line to generate the Firefly Luciferase CD22 Knockout Raji Cell Line. The PAM (Protospacer Adjacent Motif) located in exon 3 is shown in blue, an sgRNA (single guide RNA) is shown in green, and the Indel (Insertion/Deletion) in the two CD22 alleles is highlighted in red. The CD22 genomic DNA is labeled as gDNA.

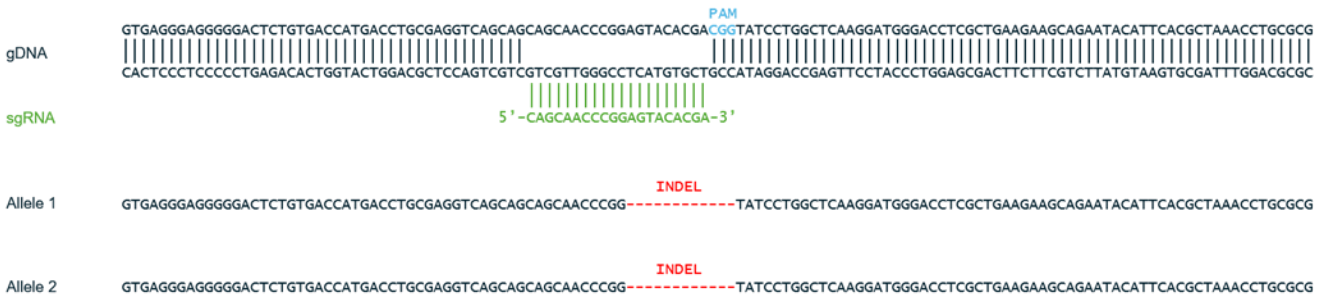

Figure 2. Genomic sequencing of CD22, exon 5, in the CD22 Knockout Raji Cell Line.
Genomic DNA from CD22 Knockout Raji cells (#82970) was isolated and sequenced. The CD22 Knockout Raji Cell Line was used as the parental cell line to generate the Firefly Luciferase CD22 Knockout Raji Cell Line. The PAM (Protospacer Adjacent Motif) located in exon 5 is shown in blue, an sgRNA (single guide RNA) is shown in green, and the Indel (Insertion/Deletion) in the two CD22 alleles is highlighted in red. The CD22 genomic DNA is labeled as gDNA.

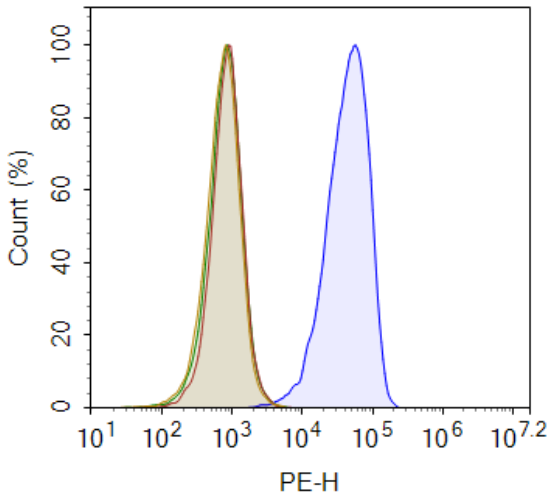

Figure 3. CD22 Expression in Firefly Luciferase CD22 Knockout Raji Cell Line by flow cytometry.
Cells were stained with PE Anti-CD22 antibody [S-HCL-1] (Abcam #ab234253) and analyzed by flow cytometry. Parental Raji cells are shown in blue, and Firefly Luciferase CD22 Knockout Raji cells are shown in red. Unstained parental Raji cells are shown in yellow. As a control, parental Raji cells were stained with PE Mouse IgG2b, κ isotype control antibody (BioLegend #400314) and shown in green. The y-axis shows the % of cells, while the x-axis represents the fluorophore intensity.

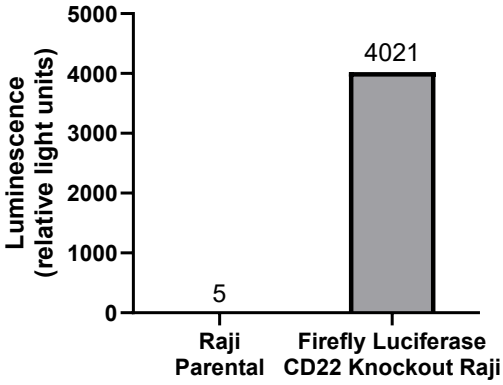

Figure 4. Luciferase activity in Firefly Luciferase CD22 Knockout Raji Cell Line.
Firefly Luciferase CD22 Knockout Raji cells and parental Raji Cells were seeded into a 96-well plate at 10000 cells/well in 100 µl of Thaw Medium 2, and the luciferase activity was measured using the ONE-Step™ Luciferase Assay System (#60690).

Data shown is representative.

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 lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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*
CD22 Knockout Raji Cell Line	82970	2 vials
CD19/CD22 Double Knockout Raji Cell Line	82956	2 vials
Firefly Luciferase CD19/CD22 Double Knockout Raji Cell Line	82969	2 vials
Anti-CD22 CAR-T Cells	78612	1 vial
Anti-CD22 CAR Lentivirus (Clone m971 ScFv-CD8-4-1BB-CD3ζ)	78608	50 µl

Version 251015