Description

Firefly Luciferase CD19 Knockout Raji Cell Line is a Raji cell line constitutively expressing firefly (*Photinus pyralis*) luciferase under the control of a CMV promoter, where CD19 (Cluster of Differentiation 19, B-lymphocyte surface antigen B4, or CVID3) has been genetically removed using CRISPR/Cas9 genome editing. This cell line was generated by using Firefly Luciferase Lentivirus (BPS Bioscience #79692) on CD19 Knockout Raji Cell Line (BPS Bioscience #82166).

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. The signal generated by the firefly luciferase reporter is proportional to Raji cell numbers and facilitates the quantification of Raji cell killing upon co-culture with CAR-T or NK cells.

CD19 (also known as Cluster of Differentiation 19, B-lymphocyte surface antigen B4, or CVID3) is a glycoprotein expressed at the surface of B lymphocytes through most phases of B cell maturation. It is strictly required for B cell terminal differentiation. Mutations in the CD19 gene cause severe immune-deficiency syndromes associated with impaired antibody production, such as CVID3 (common variable immuno-deficiency 3). The majority of B cell malignancies express normal to high levels of CD19, making it a nearly ideal target for cancer immunotherapy. Blinatumomab, a CD19/CD3 bi-specific T cell engager (BiTE) has been approved for relapsed/refractory B precursor ALL (Acute lymphoblastic leukemia) and CD19 was the target of the first approved CAR-T cell therapy. Studies of CD19 function and expression profiles will continue to broaden our knowledge and support broader applications in cancer therapy.

Application

- Use as a 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)

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. Media components are provided in the Media Formulations section.

Materials Required for Cell Line Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2E	BPS Bioscience #79638

Materials Used in Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2E	BPS Bioscience #79638
Anti-CD19 Antibody, PE-Labeled	BPS Bioscience #101625
96-well Tissue Culture-treated White Clear-bottom Assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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.

Note: Thaw Medium does *not* contain selective antibiotics. However, Growth Medium *does* 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 Line Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Growth Medium 2E (BPS Bioscience #79638):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 µg/ml of Puromycin Dihydrochloride.

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.
   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% 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 Growth Medium 2E.

Cell Passage

Dilute the cell suspension into new culture vessels at a minimum of 0.2 x 10^6 cells/ml in Growth Medium 2E. The recommended sub-cultivation ratio is 1:5 to 1:10 once or twice a 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.

Validation Data

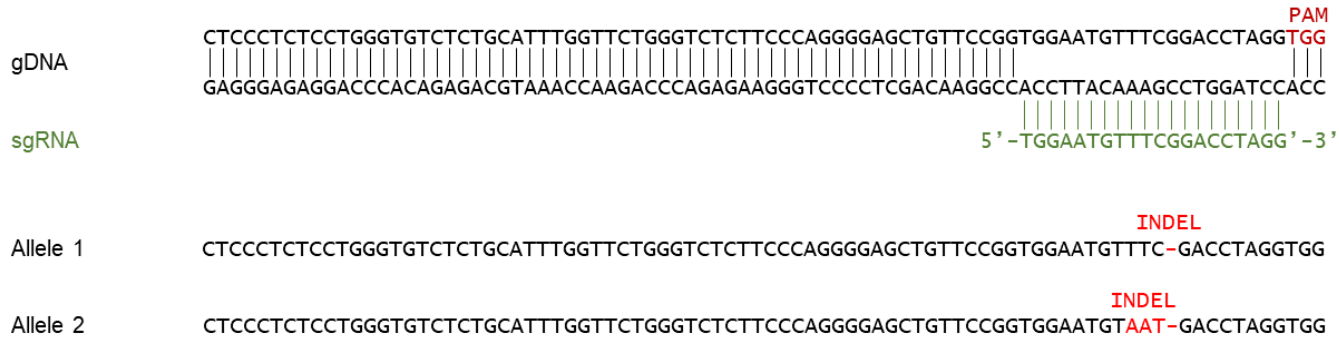

Figure 1. Genomic sequencing of CD19 in the Firefly Luciferase CD19 Knockout Raji Cell Line. Genomic DNA from Firefly Luciferase CD19 Knockout Raji cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in maroon, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two Raji alleles are highlighted in red. The CD19 genomic DNA is labeled as gDNA.

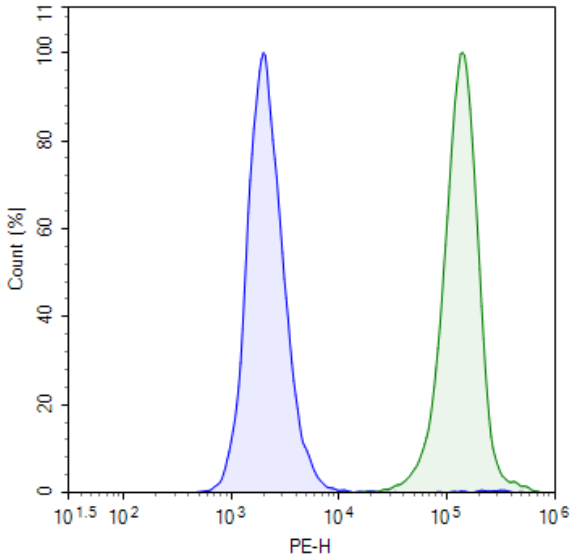

Figure 2. CD19 Expression in Firefly Luciferase CD19 Knockout Raji Cell Line.
Cells were stained with Anti-CD19 Antibody, PE-Labeled and analyzed by flow cytometry. The parental Raji cells are shown in green, and the Firefly Luciferase CD19 Knockout Raji cells are shown in blue.

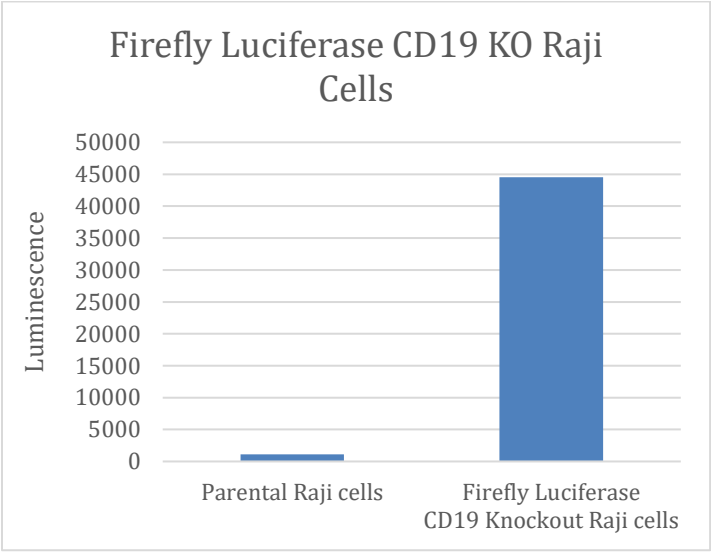

Figure 3. Luciferase activity in Firefly Luciferase CD19 Knockout Raji Cell Line.
Parental Raji cells and Firefly Luciferase CD19 Knockout Raji cells were seeded into a 96-well plate at 5,000 cells/well in 50 µl of Thaw Medium 2. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System.

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

Products	*Catalog #*	*Size*
CD19 Knockout Raji Cell Line	82166	2 vials
Cas9 Expressing Raji Cell Line	78156	2 vials
Anti-CD19 Antibody, FITC-Labeled	101863	25 µg/100 µg
Anti-CD19 IgG Antibody	100981	50 µg/100 µg
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ, eGFP)	78775	50 µl
CD19/ Firefly Luciferase CHO Cell Line	79714	2 vials

Version 110823