

Description

Firefly Luciferase Raji Cell Line is a Raji cell line constitutively expressing firefly (*Photinus pyralis*) luciferase under the control of a CMV promoter.

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 Firefly Luciferase – Raji Recombinant Cell Line makes an excellent target for CAR-T or NK cells targeting CD19, CD20, or CD22. 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 an internal 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 $>1 \times 10^6$ cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796) |

Parental Cell Line

Raji, a human B lymphoblastoid cell line derived from a patient with Burkitt's 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.

Materials Required but Not Supplied

| Name | Ordering Information |
|---|---------------------------------------|
| Thaw Medium 2 | BPS Bioscience #60184 |
| Growth Medium 2D | BPS Bioscience #79639 |
| 96-well Tissue Culture-treated White Clear-bottom Assay Plate | Corning #3610 |
| ONE-Step™ Luciferase Assay System | BPS Bioscience #60690 |
| Luminometer | |

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% CO₂. 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):

RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2D (BPS Bioscience #79639):

RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 200 µg/ml of Hygromycin B.

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% 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 Growth Medium 2D.

Cell Passage

Dilute the cell suspension into new culture vessels at no less than 0.2 x 10⁶ cells/ml in Growth Medium 2D. The sub-cultivation ratio should be approximately 1:5 to 1:10 once or twice a 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

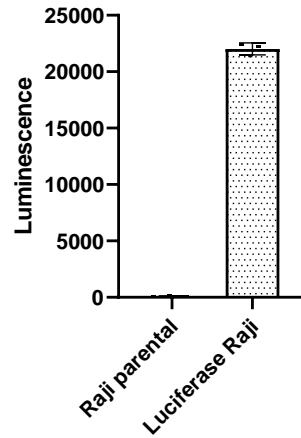


Figure 1. Luciferase activity in Firefly Luciferase Raji Cell Line.

Firefly Luciferase Raji cells were seeded into a 96-well plate at 5000 cells/well in 50 µl of Thaw Medium 2, and the luciferase activity was measured using the ONE-Step™ Luciferase Assay System (#60690).

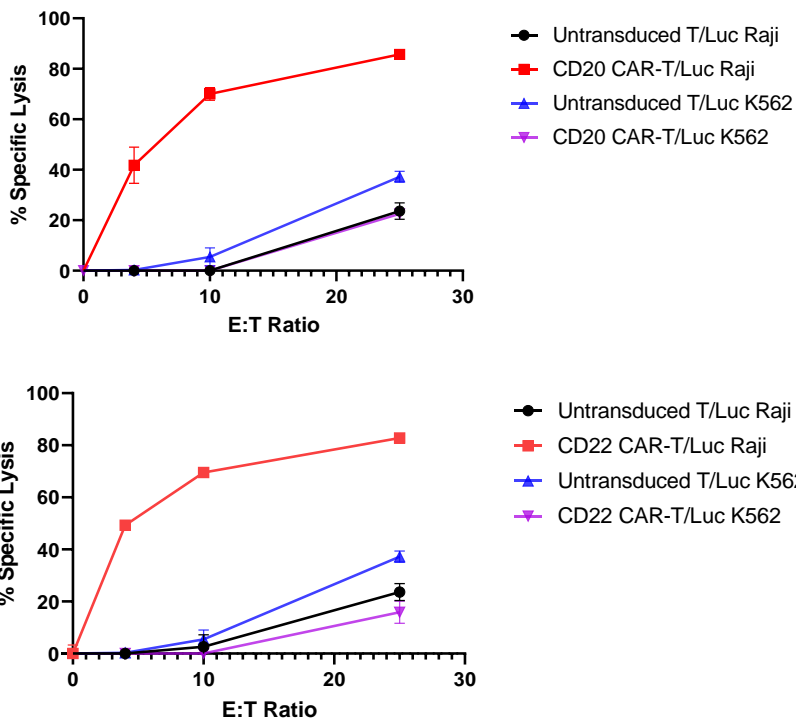


Figure 2. Cytotoxicity of Anti-CD20 or Anti-CD22 CAR-T cells against Firefly Luciferase Raji Cell Line in co-culture assays.

Firefly Luciferase Raji cells were seeded into a 96-well plate at 5000 cells/well in 50 µl of Thaw Medium 2, and cocultured overnight with 50 µl of anti-CD20 CAR-T cells (prepared using Anti-

CD20 CAR Lentivirus, #78606) or anti-CD22 CAR-T cells (prepared using Anti-CD22 CAR Lentivirus, #78608) at various effector-to-target ratios. The luciferase activity was measured using the ONE-Step™ Luciferase Assay System (#60690). Wells containing only target cells were used to determine the maximum luminescence signal (L_{max}). Wells containing only medium (no cells) was used as blank, and its luminescence was subtracted from all values. Percent specific lysis was calculated as: $[1-(L)/(L_{max})]*100$, where L corresponds to the luminescence value of the test conditions. Firefly Luciferase K562 cells (#78621) which do not express endogenous CD20 or CD22, were used as a negative control.

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.

Related Products

| <i>Products</i> | <i>Catalog #</i> | <i>Size</i> |
|---|------------------|-------------|
| Firefly Luciferase K562 Cell Line | 78621 | 2 vials |
| RPMI 8226 Recombinant Cell Line | 79834 | 2 vials |
| CD19/ Firefly Luciferase - CHO Recombinant Cell Line | 79714 | 2 vials |
| CD22/ Firefly Luciferase - CHO Recombinant Cell Line | 79715 | 2 vials |
| CD19/CD20/ Firefly Luciferase - CHO Recombinant Cell Line | 78186 | 2 vials |
| BCMA/CD20/ Firefly Luciferase - CHO Recombinant Cell Line | 78185 | 2 vials |

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