

Description

Recombinant Raji cells 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

1. Use as an internal control in CAR-T or NK co-culture killing assays
2. *In vitro* and *in vivo* Bioluminescence Imaging

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2×10^6 cells in 1 ml of 10% DMSO

Parental Cell Line

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

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, 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. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2 . 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):

RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2D (BPS Bioscience #79639):

RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 200 $\mu\text{g/ml}$ of Hygromycin B.

Cell Culture Protocol

Cell Thawing

- 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 (**no Hygromycin B**).
Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
- Immediately spin down the cells at $300 \times g$ for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2 (**no Hygromycin B**).
- Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.
- Cells should be passaged before they reach the density of 2×10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2D (**contains Hygromycin B**).

Cell Passage

Dilute the cell suspension into new culture vessels at no less than 0.2×10^6 cells/ml of Growth Medium 2D (**contains Hygromycin B**). The sub-cultivation ratio is approximately 1:5 to 1:10 once or twice a week, so cells are maintained between 0.2×10^6 cells/ml and 2×10^6 cells/ml.

Cell Freezing

- Spin down the cells at $300 \times g$ 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.
- 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.
- 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.

Validation Data

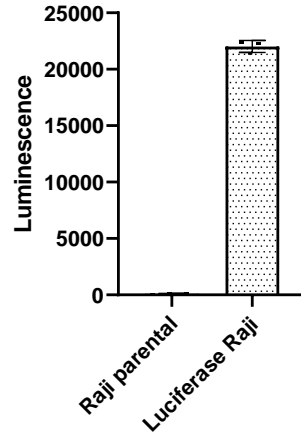


Figure 1. Luciferase activity in Firefly Luciferase Raji recombinant cells. Firefly Luciferase Raji recombinant cells were seeded into a 96-well plate at 5000 cells/well in 50 µl Thaw Medium 2, and the luciferase activity was measured using the ONE-Step luciferase assay system (BPS Bioscience, #60690).

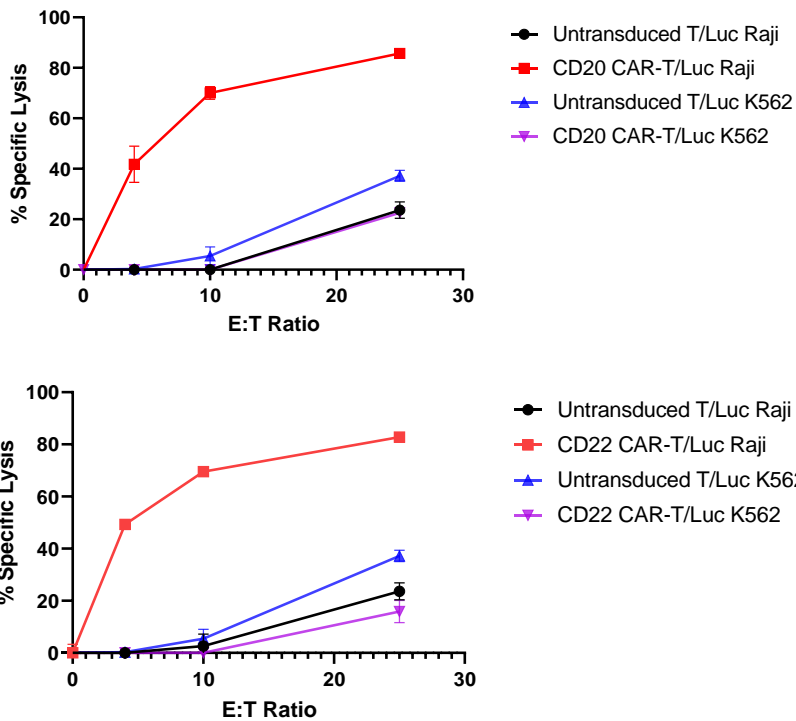


Figure 2. Anti-CD20 or anti-CD22 CAR-T cells cytotoxicity against Firefly Luciferase Raji recombinant cell line in co-culture assays. Firefly Luciferase Raji recombinant cells were seeded into a 96-well plate at 5000 cells/well in 50 µl of Thaw Medium 2, and were cocultured overnight with 50 µl of anti-CD20 CAR-T cells (prepared using anti-

CD20 CAR lentivirus, BPS Bioscience #78606) or anti-CD22 CAR-T cells (prepared using anti-CD22 CAR lentivirus, BPS Bioscience #78608) at various effector-to-target ratios. The luciferase activity was measured using the ONE-Step luciferase assay system (BPS Bioscience #60690). Target only wells determined the maximum signal (Lmax). The blank was determined from medium only wells and was subtracted from all values. Percent specific lysis was calculated as: $1-(L)/(L_{max})$. Firefly Luciferase K562 cells (BPS Bioscience #78621) which do not express endogenous CD20 or CD22, were used as a negative control.

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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

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Firefly Luciferase K562 Cell Line	78621	2 vials
Firefly Luciferase - CHO Recombinant Cell Line	79725	2 vials
RPMI 8226 Recombinant Cell Line	79834	2 vials
CD19 CAR Lentivirus	78600	50 µl
CD20 CAR Lentivirus	78606	50 µl
CD22 CAR Lentivirus	78608	50 µl
BCMA CAR Lentivirus	78603	50 µl
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