Description
Recombinant K562 cells constitutively expressing firefly (*Photinus pyralis*) luciferase under the control of a CMV promoter.

Background
The K562 cell line was established from a patient with chronic myelogenous leukemia (CML) in terminal blast crisis. The Firefly Luciferase K562 Cell Line does not express antigens CD19, CD20 or CD22, therefore it makes an excellent negative control in assays that measure specific killing by CAR-T cells targeting B cell antigens CD19, CD20, and CD22. It can also be used as a highly sensitive *in vitro* target for the natural killer assay. The signal generated by the firefly luciferase reporter is proportional to cell numbers.

Application

- *In vitro* and *in vivo* Bioluminescence Imaging
- Use as a negative control in CAR-T or NK co-culture killing assays

Materials Provided

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

Host Cell
K562, a human myelogenous leukemia cell line derived from a patient with CML; 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.

Media Required for Cell Culture

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 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 **(no Hygromycin)**.
   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 **(no Hygromycin)**.
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 **(no Hygromycin),** 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 10^6. At first passage and subsequent passages, use Growth Medium 2B **(contains Hygromycin).**

Cell Passage

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml of Growth Medium 2D (**contains Hygromycin B**). Sub-cultivation ratio: ~1:5 to1:10 once or twice a week, so cells are maintained at 0.2 x 10^6 cells/ml to 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 cells/ml.
2. 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.
3. 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

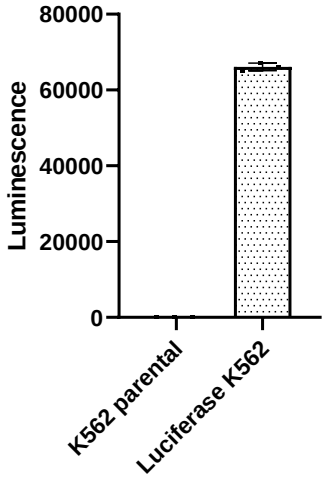

Figure 1. Luciferase activity in Firefly Luciferase K562 cells.
The cells were seeded into a 96-well plate at 5000 cells/well in 50 µl Thaw Medium 2, and the luciferase activity was measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690).

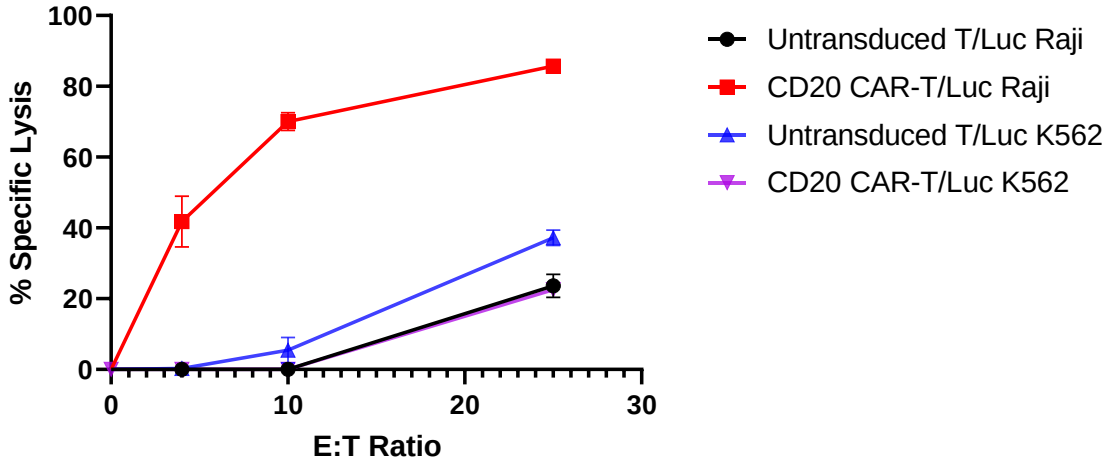

Figure 2. Lack of anti-CD20 CAR-T cytotoxicity against Firefly Luciferase K562 Cell Line.
Firefly Luciferase K562 recombinant cells, which do not express endogenous CD20, were seeded into a 96-well plate at 5000 cells/well in 50 µl Thaw Medium 2 and were cocultured overnight with 50 µl of anti-CD20 CAR-T cells (prepared using anti-CD20 CAR lentivirus, BPS Bioscience #78606) at various effector to target ratios. Firefly Luciferase Raji recombinant cells (BPS Bioscience #78622), which do express CD20 and represent *bona fide* targets for anti-CD20 CAR-T cells, were co-cultured using the same parameters. 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 and medium only wells and was subtracted from all values. Percent specific lysis was calculated as: 1-(L)/(Lmax).

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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

Products	*Catalog #*	*Size*
Firefly Luciferase Raji Recombinant Cell Line	78622	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