Description

The eGFP/Firefly Luciferase K562 Cell Line are engineered human lymphoblast K562 cells expressing firefly luciferase and enhanced GFP (eGFP) driven by an EF1a promoter. The cells were generated by transduction with Firefly Luciferase-eGFP Lentivirus (BPS Bioscience #78741), which is a SIN (self-inactivating) lentivirus.

Background

The K562 cell line was established from a patient with chronic myelogenous leukemia (CML) in terminal blast crisis. The 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 in natural killer assays. The presence of eGFP and luciferase allows for easy assay readouts, making this cell line a convenient choice.

Application

- *In vitro* and *in vivo* bioluminescence imaging (BLI) and fluorescence imaging.
- Use as negative control in CAR-T cell co-culture experiments.
- Use as target cells in NK co-culture killing assays.

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

K562, human lymphoblast cell line, 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

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

Growth Medium 2D (BPS Bioscience #79639):

RPMI-1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 200 µg/ml of Hygromycin.

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 content 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 or T75 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% 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 cells/ml. Switch to Growth Medium 2D at first and subsequent passages.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, with Growth Medium 2D. The sub-cultivation ratio should maintain the cells 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.

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

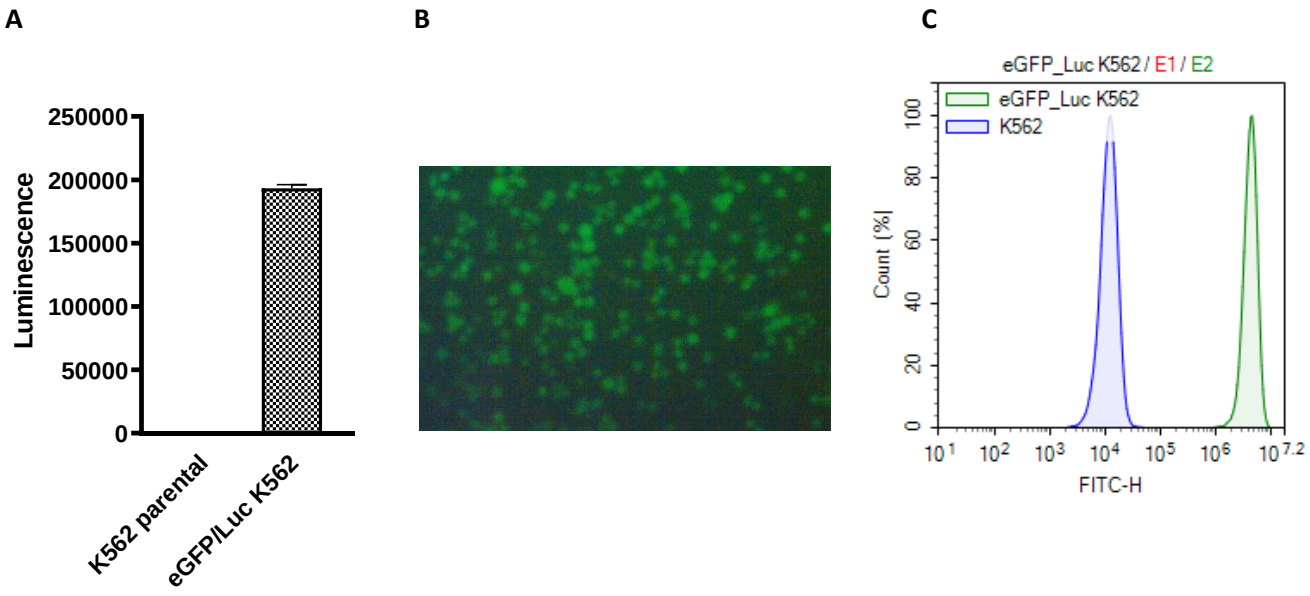

Figure 1. eGFP expression and luciferase activity in the the eGFP/Firefly Luciferase K562 Cell Line. **A.** Luciferase activity in eGFP/Firefly Luciferase K562 cells and K562 parental cells was measured using One Step™ Luciferase Assay System (BPS Bioscience #60690). **B.** Fluorescent image of eGFP/Firefly Luciferase K562 cells. **C.** 25,000 eGFP/Firefly Luciferase K562 cells and parental K562 cells were analyzed by flow cytometry for eGFP expression. The K562 Parental cells are shown in blue, and the eGFP/Firefly Luciferase K562 cells are shown in green.

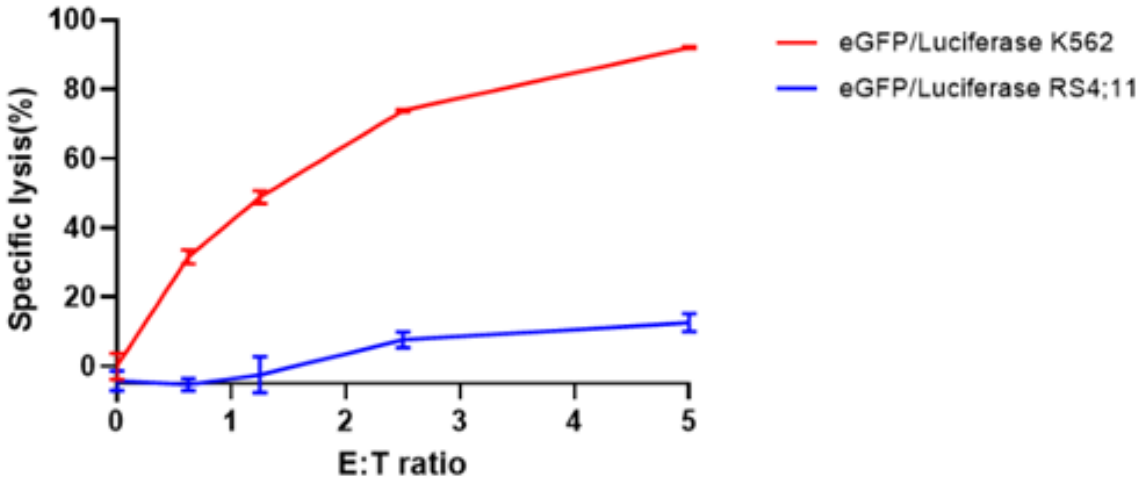

Figure 2. Luciferase activity-based NK cytotoxicity using eGFP/Luciferase K562 and eGFP/ Luciferase RS4;11 as target cells.
NK cells were enriched and expanded from PBMCs using NK Expansion Kit (BPS Bioscience #78927) for 14 days. Expanded NK cells were co-cultured with eGFP/Luciferase K562 target cells (red) and eGFP/Luciferase RS4;11 control cells (blue) at different Effector: Target (E:T) cell ratios at 37°C for 4 hours. Luciferase activity was detected using ONE-Step™ Luciferase. Cytotoxicity potency (specific lysis) correlates with a decrease in luciferase signal in target cells. Luminescence results were used to generate killing curves.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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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 Lentivirus	79692	500 µl x 2
Firefly Luciferase-eGFP Lentivirus	78741	500 µl x 2
eGFP/Firefly Luciferase U-87 MG Cell Line	78904	2 vials
eGFP/ Firefly Luciferase MM.1S Cell Line	78376	2 vials
eGFP/ Firefly Luciferase RS4;11 Cell Line	78926	2 vials

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