

Description

Firefly Luciferase CD19/CD20 K562 Cell Line is a K562 cell line constitutively expressing full length human CD19 (also known as B4 or CVID3, Genbank #NM_001770), CD20 (also known as MS4A1, Genbank #NM_152866), and the firefly luciferase reporter.

This cell line has been validated by flow cytometry and luciferase activity.

Background

B-lymphocyte antigen CD19 (Cluster of Differentiation 19), also known as B-Lymphocyte Surface Antigen B4 and CVID3, is a transmembrane protein expressed in follicular dendritic cells and all B lineage cells except plasma cells. CD19 plays two major roles in human B cells. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and it works within the CD19/CD21 complex to decrease the threshold for B cell receptor signaling pathways. Due to its presence on all B cells, it is a biomarker for B lymphocyte development and lymphoma diagnosis and can be used as a target for leukemia immunotherapies. CD19-targeted therapies based on T cells that express CD19-specific chimeric antigen receptors (CARs) have been utilized for their antitumor abilities in patients with CD19+ lymphoma and leukemia, such as Non-Hodgkins Lymphoma (NHL), CLL and ALL.

CD20 (MS4A1) is a glycosylated phosphoprotein expressed on the cell surface of B cells. CD20 is a highly attractive target antigen for immunotherapy because it is highly expressed in more than 90% of patients with B-cell lymphoma. First approved in 1997, Rituximab (Rituxan) is a chimeric monoclonal antibody targeting CD20 and has been classified by the World Health Organization as an “Essential Medicine”. Since then, additional monoclonal antibodies against CD20 have been approved or are being tested in clinical trials for the treatment of multiple sclerosis (MS), chronic lymphocytic leukemia (CLL), follicular lymphoma, diffuse large B cell lymphoma (DLBCL), rheumatoid arthritis, non-Hodgkin’s lymphoma, systemic lupus erythematosus, and myalgic encephalomyelitis (chronic fatigue syndrome). More recently, anti-CD20-CD19 bispecific CAR-T cells have been developed to address concerns over potential relapse.

Application

- Use as target cells in CAR-T or NK cell 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)

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.

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2V	BPS Bioscience #83551

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 2V (BPS Bioscience #83551):

RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 µg/ml of Puromycin and 100 µg/ml Hygromycin B.

Cell Culture Protocol

Note: K562 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 48-72 hours of culture, check for cell viability, change to fresh 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 cells/ml. Switch to Growth Medium 2V at first and subsequent passages.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2×10^6 cells/ml, with Growth Medium 2V. The sub-cultivation ratio should maintain the cells between 0.2×10^6 cells/ml and 2×10^6 cells/ml.

Cell Freezing

1. Spin down the cells at $300 \times g$ for 5 minutes. Remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of $\sim 2 \times 10^6$ 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

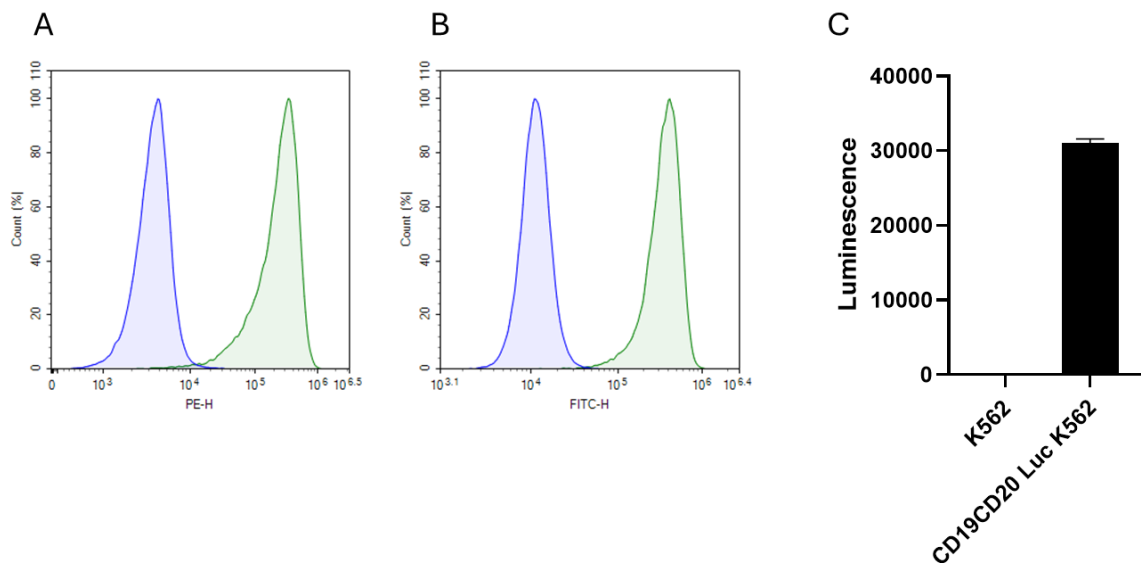


Figure 1. Expression of CD19 and CD20 and luciferase activity in Firefly Luciferase CD19/CD20 K562 Cell Line.

Firefly Luciferase CD19/CD20 K562 cells (green) or control K562 cells (blue), were labelled with FluoSite™ Anti-CD19 Antibody, PE-Labeled (#101625) (A) or FluoSite™ Anti-CD20 Antibody, FITC-Labeled (#101864) (B) and analyzed by flow cytometry. The y axis represents the % cells and the x axis indicates the fluorophore intensity. Luciferase activity in Firefly Luciferase CD19 CD20 K562 cells was measured using One Step™ Luciferase Assay System (#60690) (C).

Data shown is representative.

