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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Firefly Luciferase CD19 Knockout NALM6 Cell Line is a NALM6 cell line constitutively expressing Firefly (*Photinus pyralis*) luciferase under the control of a CMV promoter, in which CD19 (Cluster of Differentiation 19, B-lymphocyte surface antigen B4, or CVID3) has been genetically removed using CRISPR/Cas9 genome editing using a lentivirus encoding CRISPR/Cas9 gene and sgRNA (single guide RNA) targeting human CD19.

Background

NALM6 is a human B cell precursor leukemia cell line derived from the peripheral blood of a patient with acute lymphoblastic leukemia. NALM6 is a unique cell line that contains features that allow for highly efficient gene targeting via homologous recombination, the process of random gene shuffling, or nucleotide cross-over. The cell line also contains a near-diploid karyotype and is easy to transfect, making NALM6 an ideal model for knock-out or knock-in studies of gene functions. The signal generated by the Firefly luciferase is proportional to cell numbers.

CD19 (also known as Cluster of Differentiation 19, B-lymphocyte surface antigen B4, or CVID3) is a glycoprotein expressed at the surface of B lymphocytes through most phases of B cell maturation. It is strictly required for B cell terminal differentiation. Mutations in the CD19 gene cause severe immune-deficiency syndromes associated with impaired antibody production, such as CVID3 (common variable immuno-deficiency 3). The majority of B cell malignancies express normal to high levels of CD19, making it a nearly ideal target for cancer immunotherapy. Blinatumomab, a CD19/CD3 bi-specific T cell engager (BiTE) has been approved for relapsed/refractory B precursor ALL (Acute lymphoblastic leukemia) and CD19 was the target of the first approved CAR-T cell therapy. Studies of CD19 function and expression profiles will continue to broaden our knowledge and support broader applications in cancer therapy.

Application(s)

- Use as negative control in CAR-T or NK co-culture killing assays.
- *In vitro* and *in vivo* bioluminescence imaging.
- Study phenotype changes related to CD19 knockout.
- Introduce further CRISPR/Cas9-based genetic manipulations in order to understand the interplay between CD19 and other partners and pathways.

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

NALM6, human precursor B cell lymphoblasts derived from a patient with acute lymphoblastic leukemia, 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

Materials Required for Cellular Assay

Name	Ordering Information
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Anti-CD19 Antibody, PE-Labeled	BPS Bioscience #101625
96-well Flat Clear Bottom White Polystyrene TC-treated Microplates	Corning #3610
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% 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 B.

Cell Culture Protocol

Note: NALM6 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 2 to the conical tube containing the cells. Thaw Medium 2 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for viability. For a T25 flask, add 3-4 ml of fresh Thaw Medium 2 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they reach 1 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2D.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 1 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, in Growth Medium 2D**.** The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 1 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.

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

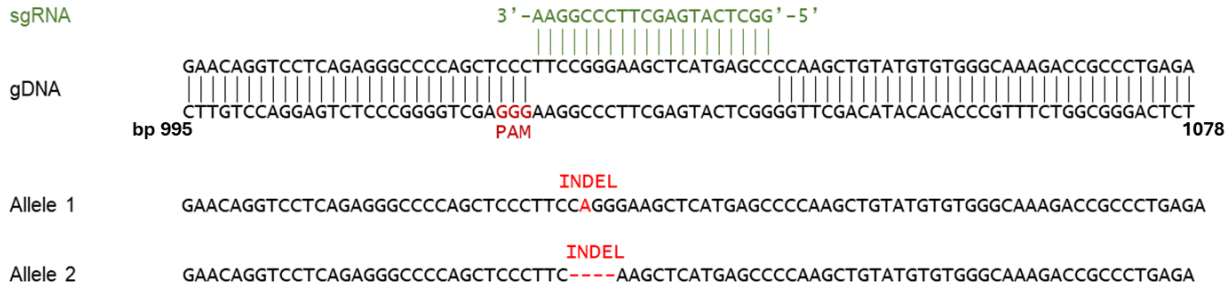

Figure 1. Genomic sequencing of CD19 in the Firefly Luciferase CD19 Knockout NALM6 Cell Line. Genomic DNA from Firefly Luciferase CD19 Knockout NALM6 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in maroon, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two NALM6 alleles are highlighted in red. The CD19 genomic DNA is labeled as gDNA.

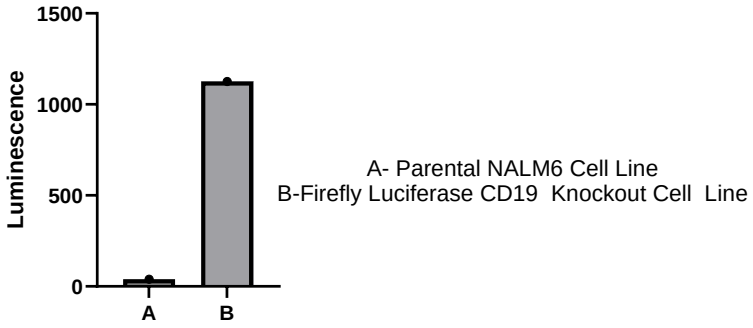

Figure 2. Luciferase activity in Firefly Luciferase CD19 Knockout NALM6 Cell Line.
Parental NALM6 and Firefly Luciferase CD19 Knockout NALM6 cells were seeded into a 96-well plate at 2.68 x 10^5 cells/well in 100 µl Thaw Medium 2, and luciferase activity was measured using the ONE-Step™ Luciferase Assay System.

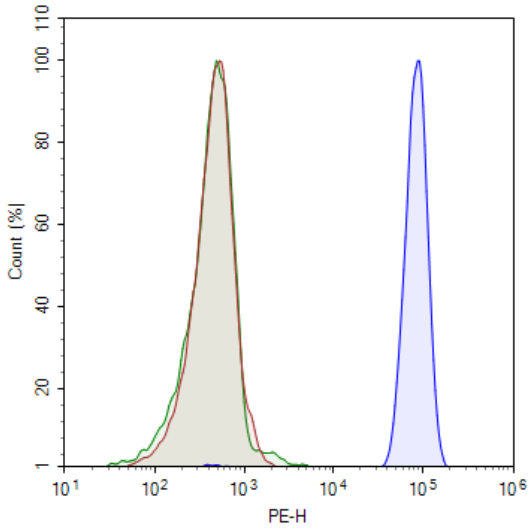

Figure 3. CD19 expression in Firefly Luciferase CD19 Knockout NALM6 Cell Line by flow cytometry.
Cells were stained with PE-Labeled Anti-CD19 Antibody (BPS Bioscience #101625) and analyzed by flow cytometry. Parental NALM6 cells are shown in blue, the Firefly Luciferase CD19 Knockout NALM cells are shown in red, and unstained parental NALM6 cells are shown in green. The Y-axis represents the % cell number. The X-axis indicates PE intensity.

References

Li Y., Zuo C., Gu L., 2021*Cancer Cell Int*. 21(1):623.
Adachi N., Nishijima H., Shibahara K., 2008 *Biosci Trends.* 2(5):169-180.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Notes

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

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	2 vials
Firefly Luciferase-eGFP Lentivirus	79980	2 vials
Firefly Luciferase NALM6 Cell Line	78494	2 vials
Firefly Luciferase CCRF-CEM Cell Line	78495	2 vials
Firefly Luciferase KG-1 Cell Line	78493	2 vials
Firefly Luciferase NK-92 Cell Line	78400	2 vials
Firefly Luciferase SKOV-3 Cell line	78425	2 vials

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