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Lieferung & Zahlungsart

siehe unsere [Liefer- und Versandbedingungen](#)

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 Cell Line is a RPMI 8226 cell Line constitutively expressing firefly (*Photinus pyralis*) luciferase and the enhanced GFP (eGFP) cassette under the control of a CMV promoter. BCMA (B-Cell Maturation Antigen, or CD269) expression has been genetically removed using CRISPR/Cas9 genome editing. This cell line was generated by using the eGFP/Firefly Luciferase Lentivirus (BPS Bioscience #79980).

This cell line has been validated by genome sequencing, flow cytometry and luciferase activity measurement.

Background

B-Cell Maturation Antigen (BCMA), also known as CD269, is a cell surface receptor of the TNF receptor superfamily that recognizes B-Cell Activating Factor (BAFF). BCMA is preferentially expressed on mature B-lymphocytes and Multiple Myeloma (MM) cells. BCMA is a highly attractive target antigen for immunotherapy not only because of its restricted expression in nonmalignant tissue, but also due to its almost universal expression on MM cells. Pre-clinical studies using CAR (Chimeric Antigen Receptor) T-cells targeting BCMA have demonstrated anti-MM activity, and in 2017, the FDA granted BCMA CAR T-Cell immunotherapy the breakthrough designation in treating Multiple Myeloma.

Application

Use as a control in CAR-T or NK co-culture killing assays.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains $>1 \times 10^6$ cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Host Cell

RPMI 8226, human B cells isolated from a plasmacytoma/myeloma patient, 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 this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results.

Materials Required for Cell Line Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2E	BPS Bioscience #79638

Materials Used in Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2E	BPS Bioscience #79638
96-well Tissue Culture-treated White Clear-bottom Assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon 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. 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 Line Culture

Thaw Medium 2 (BPS Bioscience #60184):

RPMI-1640 (ATCC modification) medium supplemented with 10% FBS and 1% Penicillin/streptomycin.

Growth Medium 2E (BPS Bioscience #79638):

RPMI-1640 (ATCC modification) medium supplemented with 10% FBS, 1% Penicillin/streptomycin, plus 0.5 ug/ml of Puromycin Dihydrochloride.

Cell Culture Protocol

Note: RPMI 8226 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 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.

- Cells should be passaged before they reach a density of 2×10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2E.

Cell Passage

Dilute the cell suspension into new culture vessels at a minimum of 0.2×10^6 cells/ml in Growth Medium 2E. The recommended sub-cultivation ratio is 1:6 to 1:8 once or twice per 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 Cell Freezing Medium (BPS Bioscience #79796) at a density of $\sim 2 \times 10^6$ cells/ml.
- 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

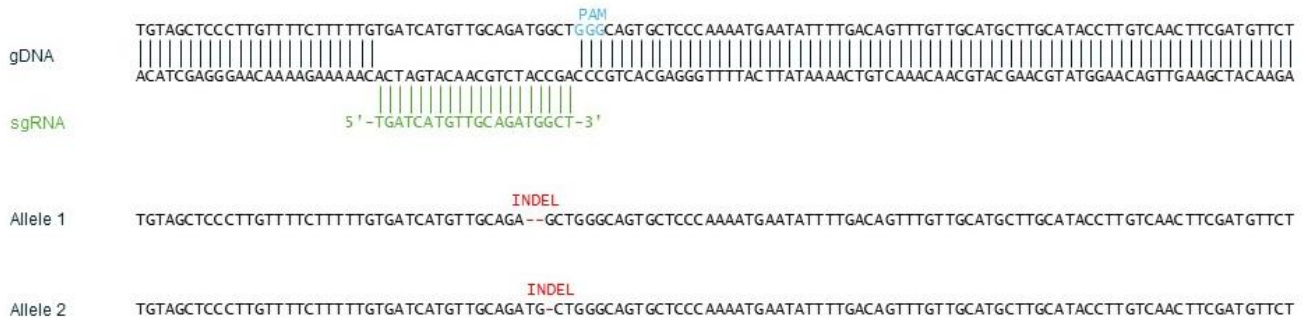


Figure 1. Genomic sequencing of BCMA in the eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 Cell Line.

Genomic DNA from the eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two BCMA alleles are highlighted in red. The BCMA genomic DNA is labeled as gDNA.

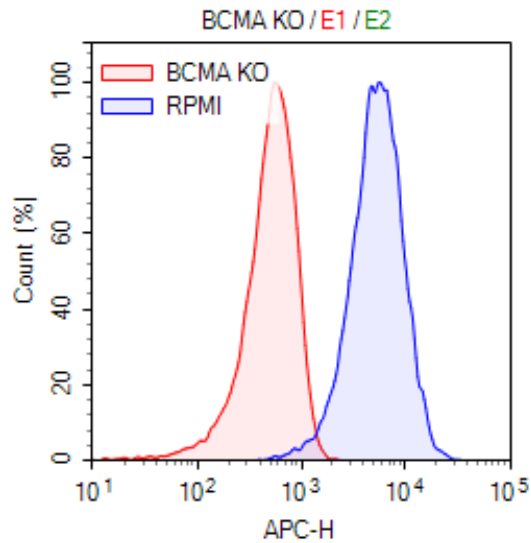


Figure 2. Flow cytometry analysis of BCMA expression in eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 Cell Line.

Cells were stained with APC-labeled anti-human CD269 (BCMA) Antibody (BioLegend #357505) and analyzed by flow cytometry. RPMI 8226 cells are shown in blue, and the eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 cells are shown in red. The y axis shows the % of cells, while the x axis represents the fluorophore intensity.

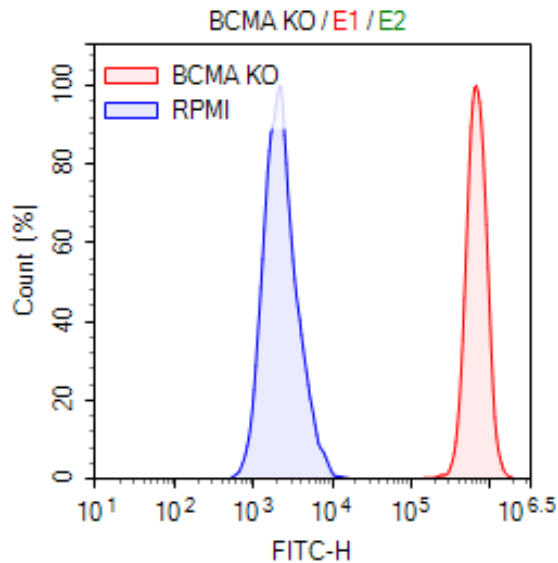


Figure 3. Flow cytometry analysis of GFP expression in eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 Cell Line.

Cells were analyzed by flow cytometry for GFP (FITC channel) expression. RPMI 8226 cells are shown in blue, and the eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 cells are shown in red. The y axis shows the % of cells, while the x axis represents the fluorophore intensity.

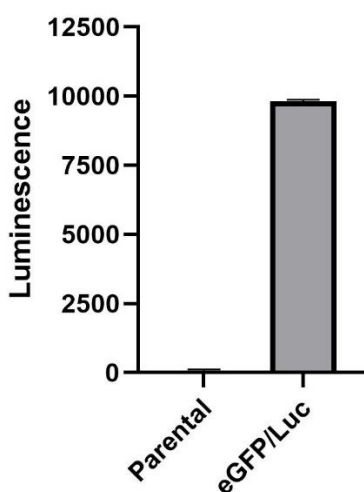


Figure 4. Luciferase activity in Firefly Luciferase BCMA Knockout RPMI 8226 Cell Line.

RPMI 8226 cells and eGFP/Firefly Luciferase BCMA Knockout RPMI 8226 cells were seeded into a 96-well plate at 5,000 cells/well in 50 μ l of Thaw Medium 2. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System.

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

License Disclosure

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

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

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.

Related Products

Products	Catalog #	Size
BCMA Knockout RPMI-8226 Cell Line	82659	2 vials
Firefly Luciferase BCMA Knockout RPMI 8226 Cell Line	82689	2 vials
BCMA Knockout MM.1S Cell Line	82687	2 vials
Anti-BCMA CAR-T Cells	78660	1 vial/5 vials
BCMA CRISPR/ Cas9 Lentivirus (Integrating)	78893	500 μ l x 2
BCMA CHO Recombinant Cell Line (High or Low Expression)	79500	2 vials
BCMA/ Luciferase – CHO Recombinant Cell Line	79724	2 vials

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