SZABO SCANDIC

Part of Europa Biosite

# Produktinformation

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

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


**SZABO-SCANDIC HandelsgmbH**

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

**Description**

Firefly Luciferase BCMA Knockout RPMI-8226 Cell Line is a RPMI-8226 cell Line constitutively expressing firefly (*Photinus pyralis*) luciferase under the control of a CMV promoter, and in which BCMA (B-Cell Maturation Antigen, or CD269) has been genetically removed using CRISPR/Cas9 genome editing. This cell line was generated by using Firefly Luciferase Lentivirus (BPS Bioscience #79692).

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 x $10^6$ 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% $CO_2$. 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 µg/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% $CO_2$ 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% $CO_2$ 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 2E.

*Cell Passage*

Dilute the cell suspension into new culture vessels at a minimum of 0.2 x $10^6$ 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 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.

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**

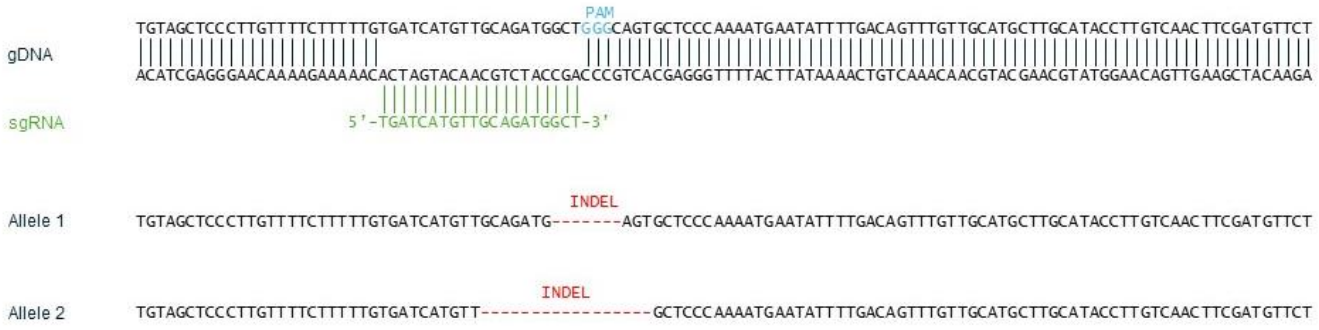


*Figure 1. Genomic sequencing of BCMA in the Firefly Luciferase BCMA Knockout RPMI-8226 Cell Line.* Genomic DNA from Firefly Luciferase BCMA Knockout RPMI-8226 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two BCMA alleles are highlighted in red. The BCMA genomic DNA is labeled as gDNA.

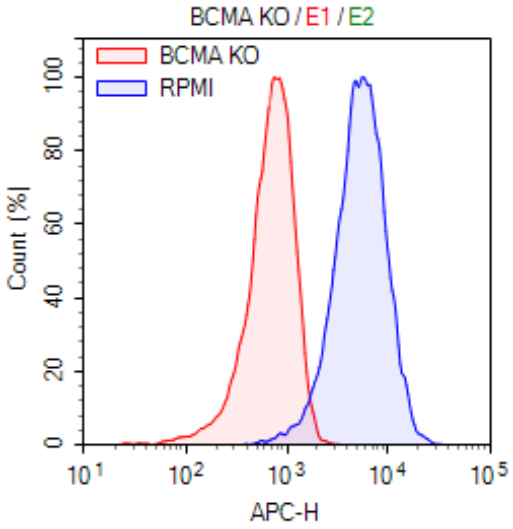


*Figure 2. Flow cytometry analysis of BCMA expression in Firefly Luciferase BCMA Knockout RPMI-8226 Cell Line.*
Cells were stained with APC anti-human CD269 (BCMA) Antibody (BioLegend #357505) and analyzed by flow cytometry. The parental RPMI-8226 cells are shown in blue, and the Firefly Luciferase BCMA Knockout RPMI-8226 cells are shown in red. The y axis shows the % of cells, while the x axis represents the fluorophore intensity.

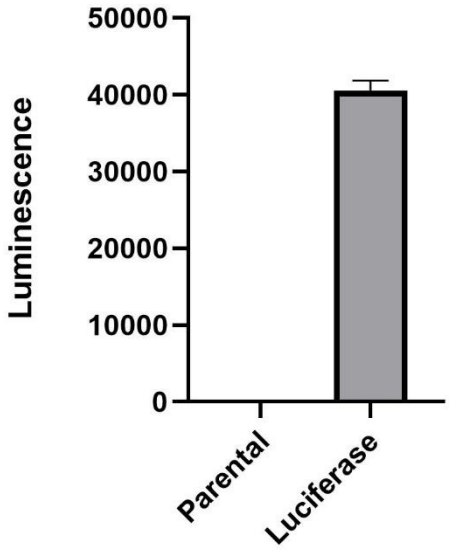


*Figure 3. Luciferase activity in Firefly Luciferase BCMA Knockout RPMI-8226 Cell Line.*
Parental RPMI-8226 cells (parental) and Firefly Luciferase BCMA Knockout RPMI-8226 cells (Luciferase) were seeded into a 96-well plate at 5,000 cells/well in 50 µl of Thaw Medium 2. Luciferase activity was measured using the ONEStep™ 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.*

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| BCMA Knockout RPMI-8226 Cell Line | 82659 | 2 vials |
| eGFP/ Firefly Luciferase BCMA Knockout RPMI-8226 Cell Line | 82688 | 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 µl x 2 |
| BCMA CHO Recombinant Cell Line (High or Low Expression) | 79500 | 2 vials |
| BCMA/ Luciferase – CHO Recombinant Cell Line | 79724 | 2 vials |

*Version 092424*