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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

eGFP/Firefly Luciferase U-87 MG Cell Line are engineered human glioblastoma U-87 MG 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

U-87 MG is an epithelial cell line isolated from a malignant glioma and it is commonly used in neuroscience and glioblastoma research. The tumor suppressor gene PTEN (phosphatase and tensin homolog) is deleted in the U-87 MG glioblastoma cell line resulting in hyperactivation of the phosphoinositide 3-kinase (PI3K) pathway, which leads to sustained PI(3,4,5)P3 signaling, and thereby hyperactivation of Akt (protein kinase B) and other effectors. Therefore, PTEN-negative U-87MG glioblastoma cells are widely used to study the modulation of the PI3K/Akt/GSK-3β pathway.

Application

- Use as target cells in CAR-T or NK co-culture killing assays.
- *In vitro* and *in vivo* bioluminescence imaging studies.

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

U-87 MG, human glioblastoma cell line, adherent.

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 1	BPS Bioscience #60187
Growth Medium 1Q	BPS Bioscience #78096

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 1 (BPS Bioscience #60187):
MEM supplemented with 10% FBS, 1% non-essential amino acids, 1mM Sodium pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1Q (BPS Bioscience #78096):
MEM supplemented with 10% FBS, 1% non-essential amino acids, 1% sodium pyruvate, 1% Penicillin/Streptomycin plus 1µg/ml Puromycin.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):
MEM supplemented with 10% FBS, 1% non-essential amino acids, 1mM Sodium pyruvate, 1% Penicillin/Streptomycin.

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 contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1**.**
   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 1.

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

4. After 48-72 hours of culture, check for cell viability, change to fresh Thaw Medium 1, 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 100% confluency. Switch to Growth Medium 1Q for passage.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA following volumes recommended for the cell vessel being used.

2. Once the cells have detached, add Growth Medium 1Q and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1Q.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:4 to 1:5 weekly or twice per week.

Cell Freezing

1. After detachment, spin down the cells at 300 *x g* for 5 minutes.
2. 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.
3. Dispense 1 ml of cell suspension into each cryogenic vial.
4. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. 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

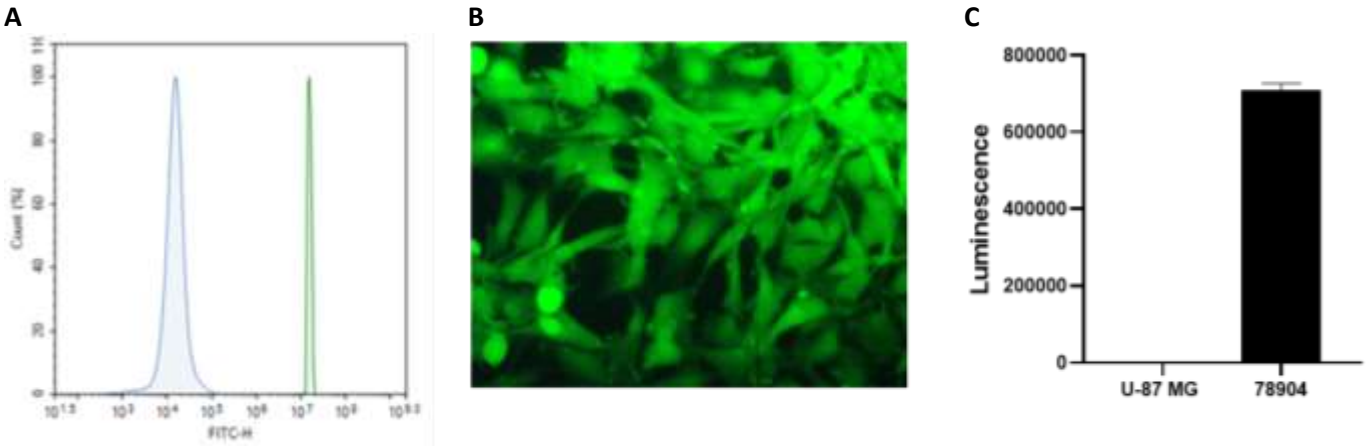

Figure 1. eGFP expression and luciferase activity in eGFP/Firefly Luciferase U-87 MG Cell Line. Panel A. eGFP expression was analyzed in eGFP/Firefly Luciferase U-87 MG cells (green) and parental U-87 MG cells (blue) by flow cytometry. Panel B. Fluorescent microscopy of eGFP/Firefly Luciferase U-87 MG cells. Panel C. Luciferase activity was measured in eGFP/Firefly Luciferase U-87 MG cells and parental U-87 MG cells using One Step™ Luciferase Assay System (BPS Bioscience #60690).

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

References

1. Allen M., *et al.*, 2016 *Sci. Trans. Med.* 8(354): 1-4.
2. Moon S.H., *et al.*, 2013 *Int J Oncol.* 42(3):921-8.

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.

Related Products

Products	*Catalog #*	*Size*
Firefly Luciferase Lentivirus (G418, Hygromycin and Puromycin)	79692	500 µl x 2
Firefly Luciferase Lentivirus EF1A Promoter/Geneticin, Hygromycin, or Puromycin)	78740	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (G418) or (Puromycin)	79980	500 µl x 2
Firefly Luciferase-eGFP Lentivirus (EF1A Promoter/Geneticin, Hygromycin, or Puromycin)	78741	500 µl x 2