Description

Recombinant MIA PaCa-2 cells expressing firefly luciferase and enhanced GFP (eGFP) driven by an EF1a promoter, generated by transduction with Firefly Luciferase-eGFP lentivirus (BPS Bioscience #78741).

Background

MIA PaCa-2 is a human epithelial cell line derived from the pancreatic cancer tissue of a 65-year-old male. It is commonly used as a tumor model in pancreatic cancer research and therapy development. The genotyping of MIA PaCa-2 confirmed the presence of a homozygous missense mutation (G12C) in KRAS (K-Ras), a homozygous deletion encompassing exons 1, 2 and 3 of the CDKN2A/p16INK4A (cyclin-dependent kinase inhibitor 2A) gene and a homozygous missense mutation (R248W) in exon 7 of TP53 (tumor protein 53, or p53).

Application

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

Materials Provided

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

Parental Cell Line

MIA PaCa-2, human pancreatic carcinoma 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 17	BPS Bioscience #78767
Growth Medium 17A	BPS Bioscience #78768

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℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 17 (BPS Bioscience #78767):
DMEM supplemented with 10% FBS, 2.5% horse serum, 1% Penicillin/Streptomycin.

Growth Medium 17A (BPS Bioscience #78768):
DMEM supplemented with 10% FBS, 2.5% horse serum, 1% Penicillin/Streptomycin plus 1 µg/ml Puromycin.

Media Required for Functional Cellular Assay

Thaw Medium 17 (BPS Bioscience #78767):
DMEM supplemented with 10% FBS, 2.5% horse serum,1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: MIA PaCa-2 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. Once 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 17.
   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 17**.**

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 in culture, check for cell viability, change to fresh Thaw Medium 17, 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 17A 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 17A 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 17A.
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. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

4. 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

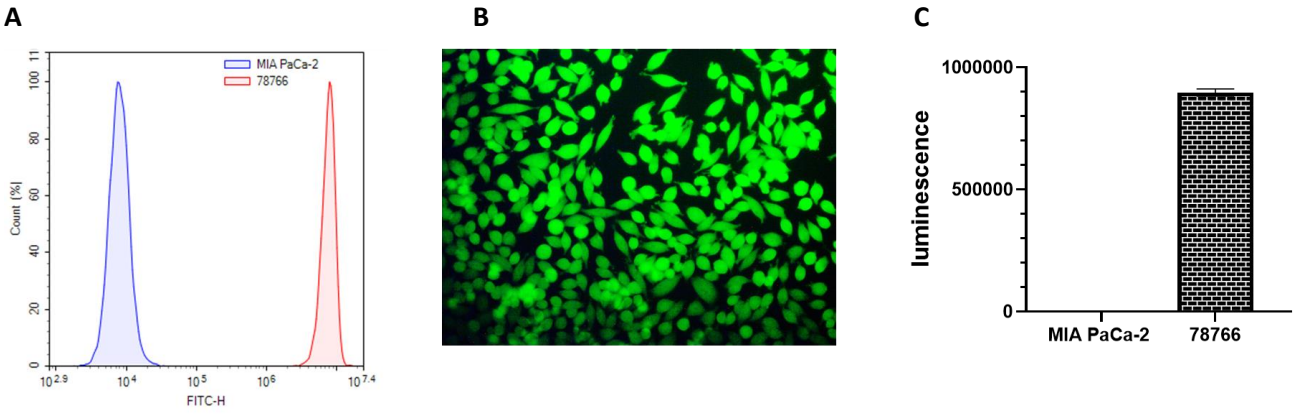

Figure 1: eGFP *expression* and Luciferase activity in GFP/Firefly Luciferase MIA PaCa-2 cells. A. eGFP expression was analysed in GFP/Firefly Luciferase MIA PaCa-2 cells (red) and MIA PaCa-2 cells (blue) by flow cytometry. B. Fluorescent microscopy of GFP/Firefly Luciferase MIA PaCa-2 cells. C. Luciferase activity in GFP/Firefly Luciferase MIA PaCa-2 cells and MIA PaCa-2 cells using One Step™ Luciferase Assay System (BPS Bioscience #60690).

References

Gradiz R, *et al*., 2016, MIA PaCa-2 and PANC-1 - pancreas ductal adenocarcinoma cell lines with neuroendocrine differentiation and somatostatin receptors. *Sci Rep.* 6:21648.

License Disclosure

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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