Description

MRC2 Knockout Firefly Luciferase A549 Cell Line is an A549 lung cancer cell line in which MRC2 (Mannose Receptor C-Type 2) is no longer expressed due to CRISPR/Cas9 genome editing. These cells also constitutively express firefly (*Photinus pyralis*) luciferase under the control of an EF1a promoter. This cell line was generated by transduction of the MRC2 Knockout A549 Cell Line (BPS Bioscience #83934) with Firefly Luciferase Lentivirus (EF1a Promoter, Hygromycin) (BPS Bioscience #78740-H).

This cell line has been validated by genomic sequencing, flow cytometry, and for luciferase activity.

Background

MRC2 (Mannose Receptor C-Type 2) is an endocytic recycling receptor that binds collagen and collagen fragments from the extracellular matrix (ECM), targeting them for lysosomal degradation. This process plays a role in extracellular matrix remodeling. As a result, MRC2 deficiency in animal models has been correlated with increased fibrosis in organs including the lung, liver, and kidney. MRC2 is also upregulated in several types of cancer in humans, including a subset of breast cancers, prostate cancers, glioblastomas, acute myeloid leukemias (AML), and sarcomas. Increased MRC2 expression may contribute to increased tumor invasion and metastasis due to the degradation of the extracellular matrix in the tumor microenvironment (TME). Given its high expression in various cancer types and potential role in driving cancer progression, it will be useful to develop antibody-drug conjugates (ADCs), CAR (chimeric antigen receptor)-T cells, or other therapies targeting MRC2.

Application

- Useful for testing MRC2-targeting therapies, including antibody-drug conjugates (ADCs) and CAR-T cells.
- Useful for studying phenotypes associated with MRC2 deficiency in a cellular model of human lung cancer.
- *In vitro* and *in vivo* bioluminescence imaging.

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

A549 is a human lung alveolar vessel carcinoma cell line. Adherent epithelial cells.

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 6	BPS Bioscience #60183
Growth Medium 6G	BPS Bioscience #83555

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. 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 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 6G (BPS Bioscience #83555)
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, 100 µg/ml Hygromycin.

Cell Culture Protocol

Note: A549 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 6.

   Notes: 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 6.

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 attachment and viability. Change medium to fresh Thaw Medium 6 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Replace media every 2-3 days until cells reach 90% confluency. At first passage and subsequent passages, use Growth Medium 6G.

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.

2. Once the cells have detached, add Growth Medium 6G 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 6G .

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. 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.

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

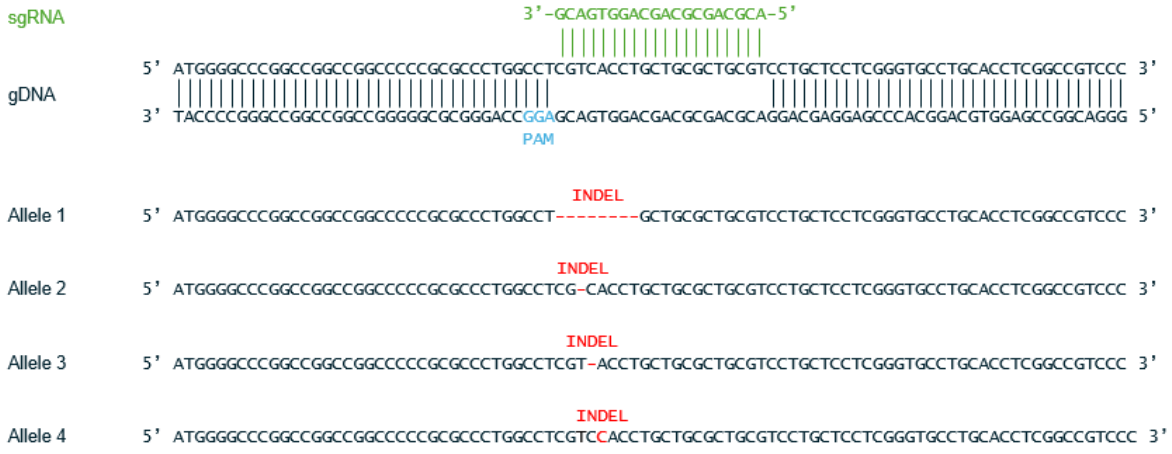

Figure 1. Genomic Sequencing of MRC2 in the MRC2 Knockout Firefly Luciferase A549 Cell Line.
Genomic DNA from MRC2 Knockout Firefly Luciferase A549 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (single guide RNA) in green, and the Indels (Insertions/Deletions) in the *MRC2* alleles are indicated in red. The *MRC2* gene is located on chromosome 17, which usually has 4 copies in A549 cells.

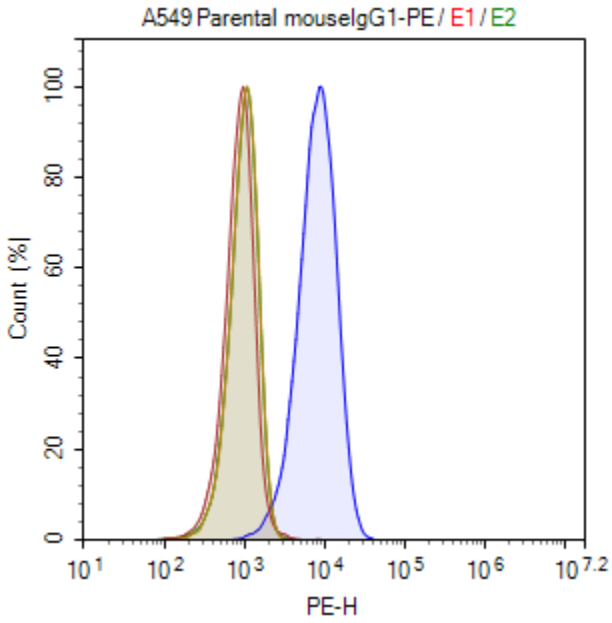

Figure 2. Expression of MRC2 in the MRC2 Knockout Firefly Luciferase A549 Cell Line by flow cytometry.
Cells were stained with PE-labeled Mouse Anti-Human CD280 (MRC2) antibody and analyzed by flow cytometry. Parental A549 cells are shown in blue, and the MRC2 Knockout Firefly Luciferase A549 cells are shown in red. Unstained A549 cells are shown in yellow. As a control, A549 cells were stained with PE-labeled Mouse IgG1, κ Isotype Control Antibody and shown in green. The y-axis shows the % of cells, while the x-axis represents the fluorophore intensity.

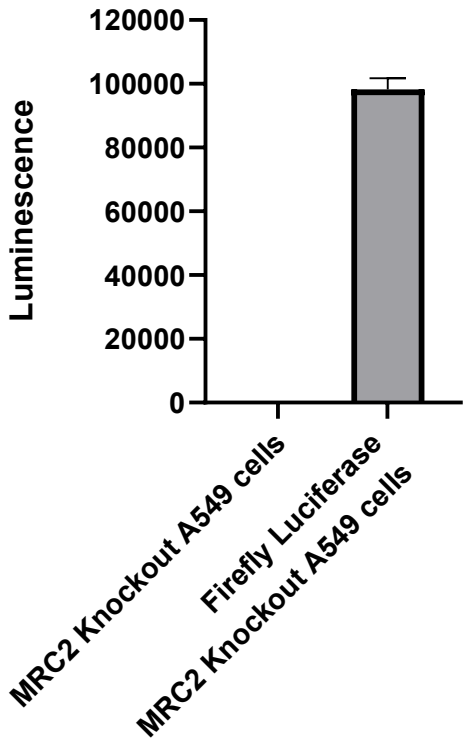

Figure 3. Luciferase activity in MRC2 Knockout Firefly Luciferase A549 Cell Line.
Parental MRC2 Knockout A549 cells and MRC2 Knockout Firefly Luciferase A549 cells were seeded into a 96-well plate in 100 µl of Growth Medium 6G. Luciferase activity was measured using the ONEStep™ Luciferase Assay System (#60690).

Data shown is representative.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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.

References

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