

Data Sheet

OSUMMER.9 Mouse NRAS-Mutant Melanoma Cell Line

Cancer Cell Line

SCC453**Pack Size: $\geq 1 \times 10^6$ viable cells/vial****Store in liquid nitrogen.****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for human or animal consumption.**

Background

Melanoma skin cancer develops from melanocytes, a cell type found in the skin that is involved in melanin production which influences skin pigmentation. While skin cancers are the most common type of cancers, melanoma is quite rare and only accounts for about 1% of skin cancer cases.¹ However, melanoma is considered a dangerous form of skin cancer due to its capability to metastasize if not diagnosed in early stages. Early-stage melanoma can be treated by resection, but fewer treatment options are available for patients with metastatic disease. Development of resistance to targeted and immunotherapies, as well as therapeutic toxicity, are major barriers to treatment. Along with sun exposure, genetic mutations have been associated with melanomas.² Immunotherapy treatments targeting these genes or pathways have been proven to have significant benefits in melanoma patients by improving general response and survival.

The OSUMMER (Ohio State University and Moffitt Melanoma Exposed to Radiation) cell lines fill a previously unfilled gap in melanoma biology. The OSUMMER cell lines are syngeneic with the C57BL/6 mouse strain and have genetic profiles that are similar to human tumors, making them responsive to immunotherapy treatments. These NRAS-mutant cell lines can be used to discover and address potential immunotherapies in NRAS-mutant tumors which make up an estimated 15-25% of human melanomas. These cell lines also enable *in vivo* testing of immunotherapies with a mouse model.

OSUMMER.9 NRAS mouse melanoma cell line was derived from a male TN mouse exposed to UVA and UVB radiation. OSUMMER.9 carries the specific NRAS mutation Q61R and can form tumors *in vivo* without Matrigel® injection. WES revealed high SNV (Single Nucleotide variants) and low CNA (Copy Number Alterations) burden, as well as the presence of UV signature mutations. OSUMMER.9 cells express the melanoma markers PMEL and MelanA and exhibit robust growth in culture.

Source

OSUMMER.9 was derived from melanoma induced in TN (Tyr::CreER) mice exposed to UVA or UVB radiation on postnatal day 3.³

Short Tandem Repeat (STR) Profile

M18-3: 16	M7-1: 26.2, 27.2	M15-3: 22.3	M12-1: 17
M4-2: 20.3	M1-1: 16	M6-4: 18	M5-5: 18
M6-7: 15	M3-2: 14	M11-2: 16	MX-1: 27
M19-2: 13	M8-1: 16	M17-2: 15	M13-1: 17
M1-2: 19	M2-1: 9, 16		

Cancer cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- Each vial contains $\geq 1 \times 10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR Panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for inter-species contamination from human, rat, Chinese hamster, Golden Syrian hamster, and Non-human Primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

OSUMMER.9 cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Representative Data

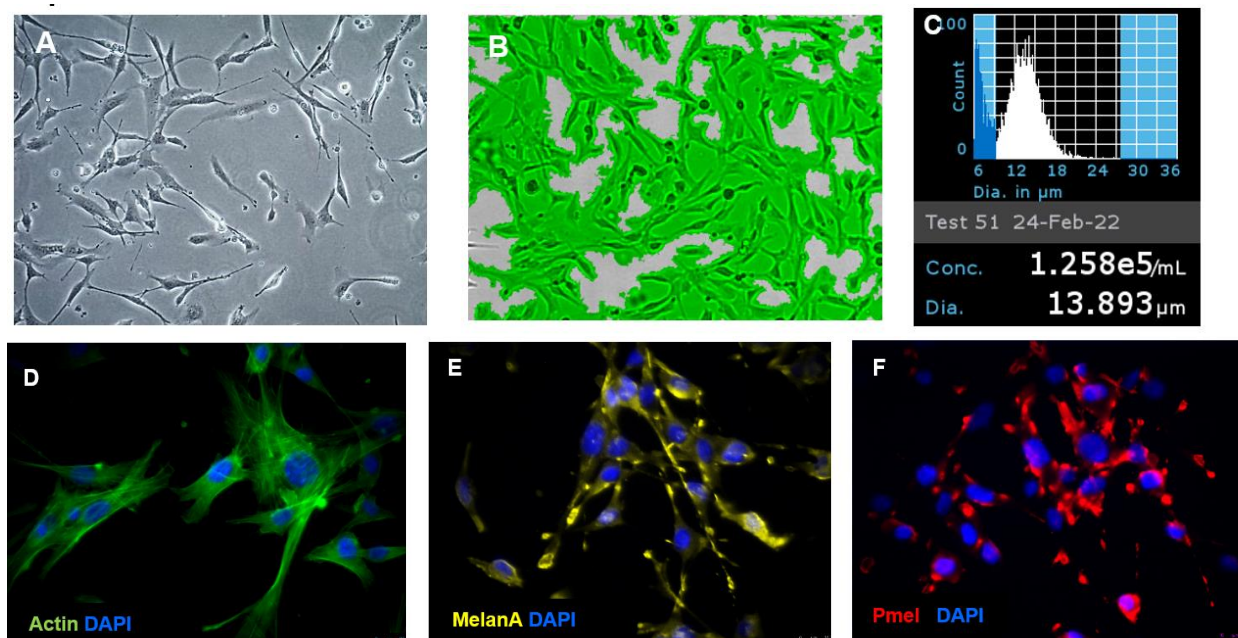


Figure 1. (A) Brightfield image of OSUMMER.9 cells two days after thaw in a T75 flask. (B, Cat. No. MDCI 10000) Cell confluency (80%) was assessed throughout the culture using Millicell® Digital Cell Imager. (C, Cat. No. PHCC360KIT) Cell counting was performed using Scepter™ 3.0 handheld automated cell counter using 60 μm sensors. (D) OSUMMER.9 cells express actin and the melanoma markers, (E) MelanA and (F) PMEL.

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.
Cells are thawed and expanded in OSUMMER Expansion Medium comprised of DMEM-High Glucose medium (Cat. No. D5796) with 10% FBS (Cat. No. ES-009-B), 2 mM L-Glutamine (Cat. No. TMS-002-C), and 1X penicillin/streptomycin (Cat. No. TMS-AB2-C, optional).
2. Remove the vial of frozen OSUMMER.9 cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.
IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1- or 2- mL pipette to transfer the cells to a sterile 50 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of OSUMMER Expansion Medium (Step 1 above) to the 15 mL conical tube.
IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.
IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of OSUMMER Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO₂.

Subculturing Cells

1. OSUMMER.9 cells should be passaged at ~80-85% confluency. Do not allow the cells to grow over 85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of OSUMMER.9 cells. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 3-5 mL of Accutase® or Trypsin/EDTA solution and incubate in a 37 °C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 8 mL of OSUMMER Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of OSUMMER Expansion Medium to the conical tube and resuspend the cells thoroughly.
IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer or a Scepter™ 3.0 handheld automated cell counter using 40 µm sensor tips.
11. Plate the cells to the desired density. Typical split ratio is 1:5 to 1:6. The medium should be replaced every other day.

Cryopreservation of Cells

OSUMMER.9 cells may be frozen in OSUMMER Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Nat Rev Dis Primers 2015, 1: 15003.
2. Lancet 2018, 392(10151): 971-984.
3. Life Sci Alliance 2021, 4(9): e202101135.

Academic Use Agreement

Subject to local law

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means OSUMMER.9 Mouse NRAS-Mutant Melanoma Cell Line (SCC453).

"Academic Research" means any internal in vitro research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

Genetically Modified Organisms (GMO)

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma, Millicell, Scepter and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
© 2008-2023 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

Document Template 20306518 Ver 6.0
00144587 Ver 1.0, Rev 19OCT2023, RC, AB

**Millipore
Sigma**