

Data Sheet

YUMM5.4 Mouse Melanoma Cell Line

Mouse Melanoma Cell Line

SCC241

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

The promise of immunotherapies in cancer and recent progress in successful application of these approaches has highlighted the necessity of immunocompetent models to evaluate immune system responses to cancer cells. Immunocompetent genetically engineered mouse models harboring particular genetic drivers of melanoma are essential for studying potential immunotherapies but are limited by the need to maintain animal colonies of multiple genotypes necessary to generate models with appropriate genetic backgrounds.

The YUMM (Yale University Mouse Melanoma) cell lines were developed as models that recapitulate genetic drivers found in a significant proportion of human melanomas.¹ YUMM5.4 cells are syngeneic with the immunocompetent C57Bl/6 mouse background and retain genetic markers of the *Braf/Pten* mouse model.¹ Approximately half of all melanomas harbor *Braf* mutations, of which the V600E mutation comprises 90% of cases, while the tumor suppressor p53 is inactivated in 90% of melanomas.² The YUMM5.4 cell line harbors the activating *Braf* V600E mutation and is negative for p53.¹ The YUMM5.4 mouse melanoma cell line represents a valuable tool for investigating tumor biology and developing therapeutic approaches for melanomas with this highly prevalent mutational profile.

Source

The YUMM5.4 mouse melanoma cell line was derived from a 4-hydroxytamoxifen-induced melanoma tumor in a male C57/Bl/6 mouse into which mutations from the *Braf/Pten* genetically engineered mouse model had been introduced via backcrossing. The YUMM5.4 cell line harbors the *Braf* V600E mutation and is homozygous negative for p53.¹

Short Tandem Repeat

M18-3: 16	M1-2: 19	M8-1: 16	M11-2: 16, 17	MX-1: 27
M4-2: 20.3	M7-1: 27.2, 28.2	M2-1: 9, 16	M17-2: 12, 16	M13-1: 17, 18
M6-7: 17, 18	M1-1: 17	M15-3: 22.3	M12-1: 17	
M19-2: 13	M3-2: 14	M6-4: 18	M5-5: 17	

Quality Control Testing

- YUMM5.4 cells are verified to be of mouse origin and negative for rat, Chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) interspecies contamination as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

YUMM5.4 cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

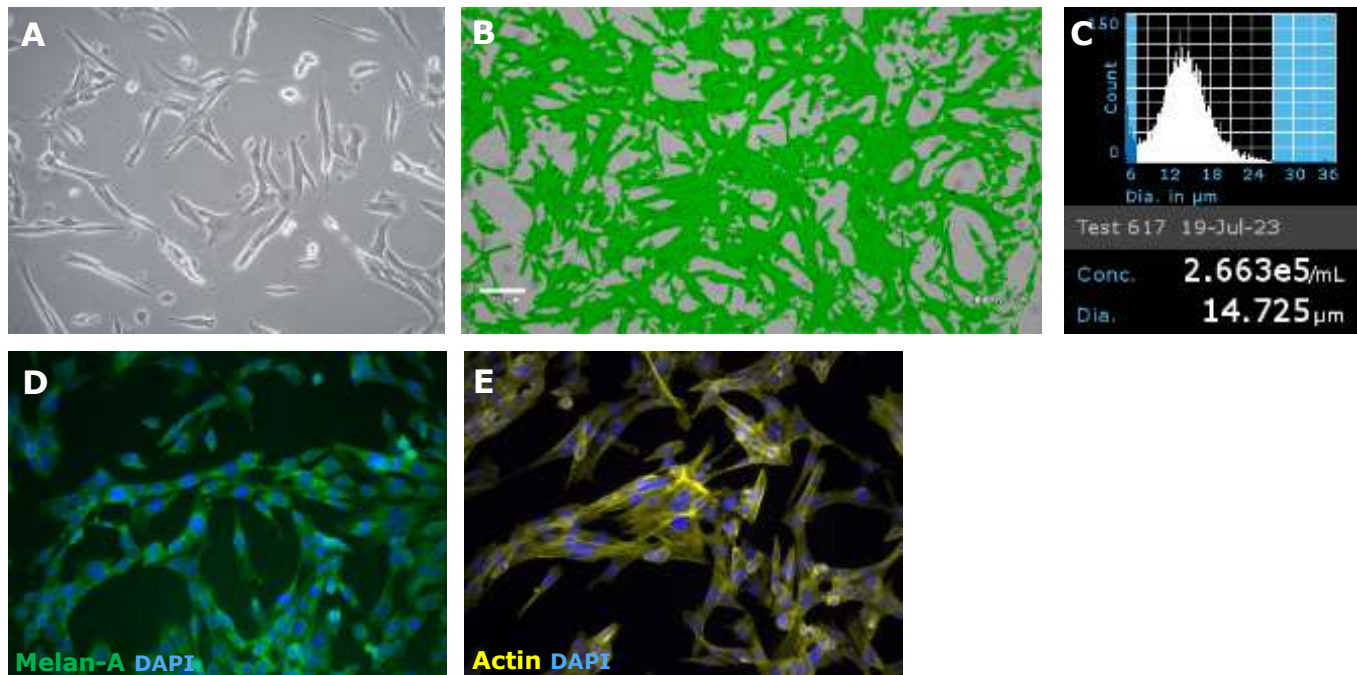


Figure 1. **A.** Bright-field image of YUMM5.4 cells one day after thaw in a T75 flask (10X Magnification). **B.** Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). **C.** Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 μm sensor tips (PHCC360KIT). **D.** YUMM5.4 cells express the melanoma marker Melan-A (Fisher Scientific, PA5-99174). **E.** YUMM5.4 cells stained with Phalloidin-Atto 565 for actin (94072).

Note: Product catalog numbers indicated in () can be purchased at [SigmaAldrich.com](https://www.sigmaaldrich.com) unless otherwise stated.

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating. Cells are thawed and expanded in YUMM Expansion Medium comprising DMEM/F12 medium (DF-042-B) containing 10% FBS (ES-009-B) and 1X non-essential amino acids (TMS-001-C).
2. Remove the vial of frozen YUMM5.4 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 15 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 YUMM 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 5 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 YUMM Expansion Medium and transfer the cell mixture to a T75 tissue culture flask.
10. Incubate the cells at 37 °C in a humidified incubator with 5% CO₂.

Subculturing the Cells

1. Do not allow the cells to grow to confluency. YUMM5.4 cells should be passaged at ~80-85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of YUMM5.4 cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 3-5 mL of Accutase® and incubate in a 37 °C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of YUMM Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of YUMM Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.
IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

YUMM5.4 cells may be frozen in YUMM Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Meeth K, Wang JX, Micevic G, Damsky W, Bosenberg MW. 2016. The YUMM lines: a series of congenic mouse melanoma cell lines with defined genetic alterations. *Pigment Cell Melanoma Res.* 29(5): 590-597.
2. Box NF, Vukmer TO, Terzian T. 2014. Targeting p53 in melanoma. *Pigment Cell Melanoma Res.* 27(1): 8-10.

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