Data Sheet

# YUMM2.2 Mouse Melanoma Cell Line

Mouse Melanoma Cell Line

**SCC234**

**Pack Size ≥ 1x10$^6$ 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 great promise of immune-based therapies in cancer and recent progress in successful application of these approaches has brought to the fore the necessity of immune-competent models to evaluate immune system responses to cancer cells. Immunocompetent genetically engineered mouse models harboring discrete genetic drivers of melanoma are essential for studying potential immunotherapies but are limited by the need to maintain colonies of multiple genotypes necessary to generate mouse 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] YUMM2.2 cells are syngeneic with the immunocompetent C57/Bl/6 mouse background and retain genetic markers of the *Braf/Pten* mouse model, characterized by activation of *Braf* and inactivation of *Pten* and Cdkn2a.[1] The YUMM2.2 cell line harbors a stabilized beta-catenin allele from excision of exon 3, a mutation that accelerates *Braf/Pten*-driven melanoma tumorigenesis, enhances metastasis, and controls tumor differentiation.[2] Exon 3 excision is analogous to human beta-catenin stabilizing mutations found in human melanomas.[2] The YUMM2.2 mouse melanoma cell line represents a valuable tool for investigating the role of beta-catenin signaling in melanomas.

## Source

The YUMM2.2 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 YUMM2.2 cell line harbors the *Braf* V600E mutation and is homozygous negative for wild-type *Pten,* heterozygous for *Cdkn2,* and characterized by expression of stable beta-catenin via partial *Cre/lox* recombination.[1]

## Short Tandem Repeat

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

## Quality Control Testing

- YUMM2.2 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 infectious diseases against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

## Storage and Handling

YUMM2.2 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

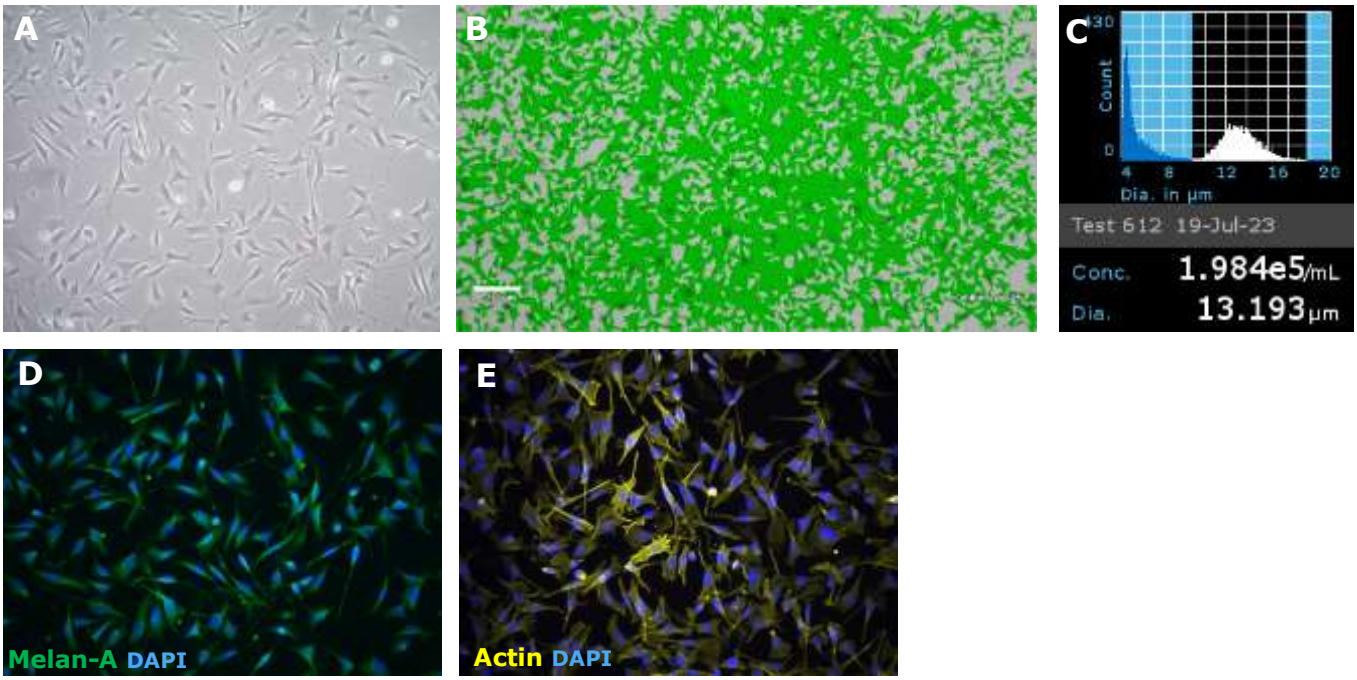


**Figure 1. A.** Brightfield image of YUMM2.2 cells one day after thaw in a T75 flask (10X Magnification). **B.** Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). **C.** Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 40 µm sensor tips (PHCC340KIT). **D.** YUMM2.2 cells express the melanoma marker Melan-A (Fisher Scientific, PA5-99174). **E.** YUMM2.2 cells stained with Phalloidin-Atto 565 for actin (94072).

**Note**: Product catalog numbers indicated in ( ) can be purchased at 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 YUMM2.2 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% $CO_2$.

### Subculturing the Cells

1. Do not allow the cells to grow to confluency. YUMM2.2 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 YUMM2.2 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

YUMM2.2 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. Damsky WE, Curley DP, Santhanakrishnan M, Rosenbaum LE, Platt JT, Rothberg BEG, Taketo MM, Dankort D, Rimm DL, McMahon M, et al. 2011. B-catenin signaling controls metastasis in Braf-activated Pten-deficient melanomas. Cancer Cell. 20(6): 741-754.


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