Data Sheet

YUMM1.4 Mouse Melanoma Cell Line

Cancer Cell Line

SCC226

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

As immune-based therapies in cancer models become more popular and applicable, a further need for immune-competent models has been presented. A wide variety of genetically engineered mouse models have been created to express vital melanoma genotypes. The variety in these models have allowed the study of genetic alterations that are relevant to human melanoma biology.[1] While it is quite useful to have mouse models with genetically defined human melanoma characteristics, it is limited by complex mouse colony maintenance.

The YUMM1.4 cell line was produced to be more experimentally relevant. The cell line was derived by backcrossing important alleles into C57BL/6J mice. The mice were interbred to produce human-relevant genetically defined mouse models that could then be used to generate cell lines. Melanomas were induced by applying 4-hydroxytamoxifen to induce Cre-lox allele recombination. Cells were then mechanically and enzymatically dissociated from the removed tumor. The melanoma lines generated from this procedure retained important human melanoma characteristics useful for studying disease progression.[1]

The YUMM1.4 cell line is driven by Braf activation, Pten inactivation, and Cdkn2a inactivation.[1] The cell line is positive for the common melanoma marker, MelanA. The cells are syngeneic with C57BL/6J mice and are very tumorigenic *in vivo*.

Source

Cell line was derived from a 4-hydroxytamoxifen-induced melanoma tumor in a male C57BL/6 mouse into which mutations from the Braf/Pten genetically engineered mouse model had been introduced via backcrossing.

Short Tandem Repeat

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

Quality Control Testing

- The YUMM1.4 Mouse melanoma cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate 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

The YUMM1.4 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

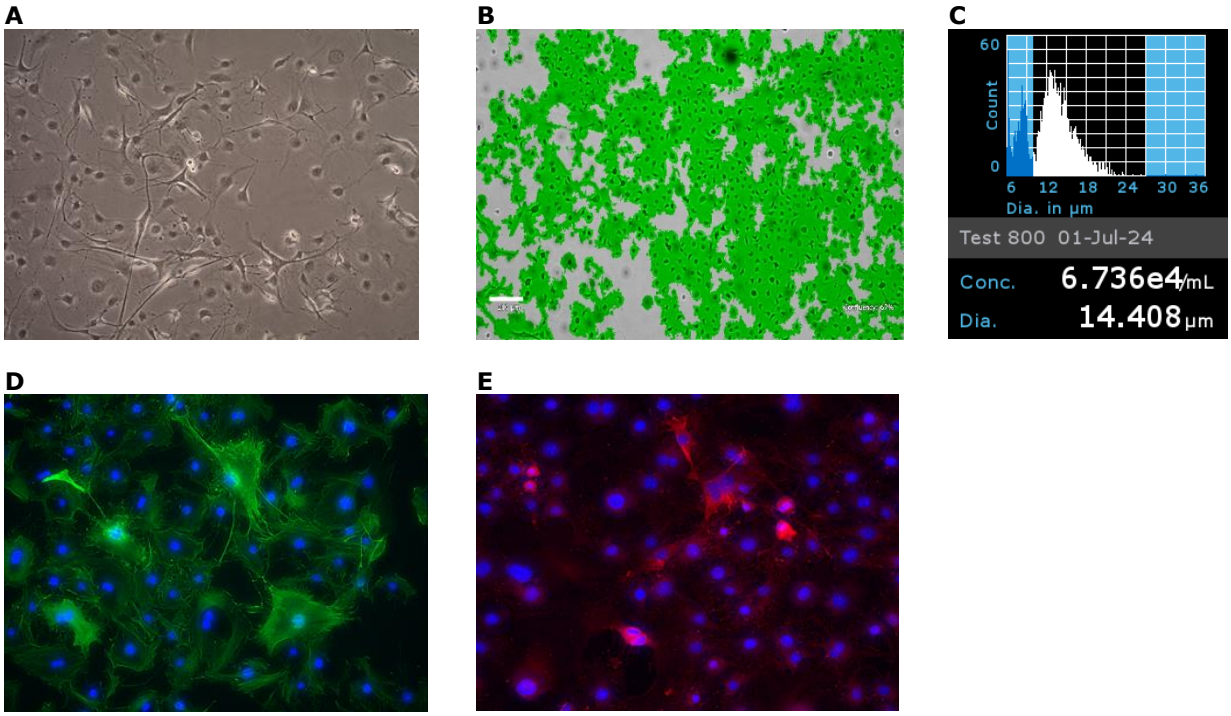

Figure 1. (**A**) Bright-field images of YUMM1.4 cells one day after thaw in a T75 flask (4X magnification). (**B**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C**) Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors (PHCC360KIT). (**D**) YUMM1.4 cells stained with Phalloidin-Atto-488 (49409). (**E**) YUMM1.4 cells express MelanA protein (ThermoFisher, MA5-37601).

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. YUMM1.4 cells are thawed and expanded in YUMM Expansion Medium comprising of DMEM/F12 (DF-042-B) containing 10% FBS (ES-009-B), 2 mM L-Glutamine (G7513), NEAA (TMS-001-C), and Penicillin/Streptomycin (P4333) (optional).
2. Remove the vial of frozen YUMM1.4 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 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 YUMM 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% CO_2.

Subculturing the Cells

1. YUMM1.4 cells can be passaged at ~80-85% confluency.
2. Carefully remove the medium from the tissue culture flask containing the 80-85% confluent layer of YUMM1.4 cells.
3. Rinse the flask with 10 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® (A6964) and incubate in a 37 °C incubator for 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 3-5 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 cell 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 1:8.

Cryopreservation of the Cells

The YUMM1.4 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, J. X., Micevic, G., Damsky, W., & Bosenberg, M. W. (2016). The YUMM lines: a series of congenic mouse melanoma cell lines with defined genetic alterations. Pigment cell & melanoma research, 29(5), 590-597.

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