Data Sheet

YUMMER1.7D5 Mouse Melanoma Cell Line

Cancer Cell Line

SCC244

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. Melanomas exhibit relatively high somatic mutation burden, and these mutations may act as neoantigens that generate anti-tumor immune responses. The development of immunocompetent cell models is critical to the advancement of cancer immunotherapy and understanding of immune responses, although few tractable model systems are available.

The YUMMER1.7D5 mouse melanoma cell line is both immunocompetent and reflective of the somatic mutations common in melanomas. YUMMER1.7D5 carries three driver mutations of melanoma: Braf V600E, Pten -/- and Cdkn2 -/-.[1,2] In addition, the YUMMER1.7D5 cell line harbors a high frequency of stable UV-induced somatic mutations which have been shown to stimulate host adaptive immune response.[3] YUMMER1.7D5 cells are diploid, allowing for enhanced knockout frequency in CRISPR-based screens. The unique features of the YUMMER1.7D5 cell line make it a valuable model for studies of immune checkpoint inhibition and mechanisms of anti-tumor responses.

Source

The YUMMER1.7D5 mouse melanoma cell line is a diploid clonal isolate from YUMM1.7D5 cells exposed to UVB radiation. The original YUMM1.7 cell line was derived from a 4-hydroxytamoxifen-induced melanoma tumor in a male C57/B1/6 mouse into which mutations from the Braf/Pten genetically-engineered mouse model had been introduced via backcrossing.[1] The YUMMER1.7D5 cell line harbors the Braf V600E mutation and is homozygous negative for wild-type Pten and Cdkn2.[3]

Storage and Handling

YUMMER1.7D5 mouse melanoma cell line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥ $1x10^6$ 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 rat, Chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Representative Data

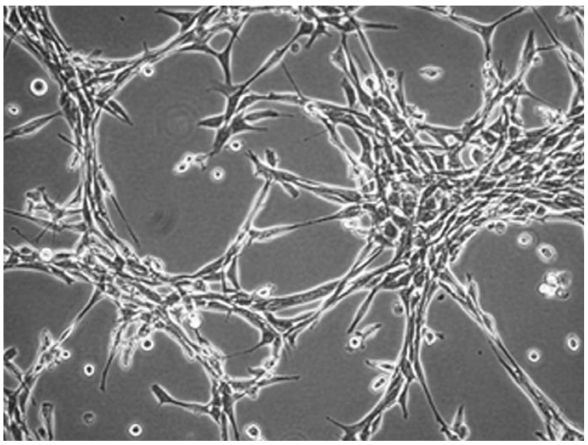

Figure 1. YUMMER1.7D5 cells two days after thawing in a T75 flask.

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.

   YUMMER1.7D5 Expansion Medium: Cells are thawed and expanded in DMEM/F12 medium (Cat. No. DF-041-B) supplemented with 10% FBS (Cat. No. ES-009-B) and 1X non-essential amino acids (Cat. No. TMS-001-C).
2. Remove the vial of frozen YUMMER1.7D5 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 YUMMER1.7D5 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.

   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.
6. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.

7. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
8. Resuspend the cells in 15 mL of YUMMER1.7D5 Expansion Medium.
9. 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 Cells

Note: Cells are more adherent when they are sparsely populated. Upon reaching a confluent monolayer, cells become more easily detach and may detach as a monolayer, even without trypsin treatment. The monolayer detachment may result in clumpy cells and thus we recommend using Accutase™ or Accumax™ instead of trypsin.

1. Inspect the flask containing the subconfluent layer of YUMMER1.7D5 cells. If cells are detached as a monolayer, collect the cell suspension into a conical tube and centrifuge to pellet the cells. Discard the supernatant from the conical tube.
2. If there are any remaining attached cells, apply 5-7 mL Accutase™ or Accumax™ to the flask and immediately pipette up and down to detach the remaining cells. It is not necessary to incubate at 37 °C for 3-5 minutes.
3. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand. Collect the detached cells and add to the cell suspension from step 1.
4. Pipette up and down to dissociate the combined cell suspension into a single cell suspension.
5. Add 5-7 mL of YUMMER1.7D5 Expansion Medium to the cell suspension.
6. Centrifuge the tube containing the cell suspension at 300 *x g* for 3-5 minutes to pellet the cells.
7. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
8. Apply 2-5 mL of YUMMER1.7D5 Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

YUMMER1.7D5 Mouse Melanoma Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container

References

1. Meeth K et al. (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. Dankort D et al. (2009) Braf (V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet. 41(5): 544-552.
3. Wang J et al. (2017) UV-induced somatic mutations elicit a functional T cell response in the YUMMER1.7 mouse melanoma model. Pigment Cell Melanoma Res 30(4): 428-435.

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