Data Sheet

D4M-3A Mouse Melanoma Cell Line

Tumor Cell Line

SCC428

Pack Size ≥ 1x10^6 viable cells/vial

Store at: Liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Although it is rarer than carcinomas, melanoma is generally considered the most serious of skin cancers due to its potential for metastasis. The BRafV600E mutation, which occurs in nearly 50% of human melanomas, constitutively activates pERK, a component of the MAP kinase signaling pathway, and contributes to disease progression.[2] Although human melanoma cell lines harboring the BRafV600E mutation are well-established, the reliance on xenografts in immunocompromised mice limits studies of host-tumor cell interactions in a syngeneic background.[2]

The D4M-3A cell line is a melanoma cell line from the conditional mouse model of metastatic melanoma: Tyr::CreER, BrafCA;Ptenlox/lox, which recapitulates human disease. Cultured D4M-3A cells express high constitutive pERK and respond to the BRafV600E inhibitor, Vemurafenib (PLX4032).[2] D4M-3A cells lack melanin expression but maintain the ability to express melanocyte differentiation antigens, which are important immune recognition factors. D4M-3A cells are transplantable in either immune-compromised or syngeneic B6 mice and show correlation of *in vitro* studies on molecular mechanisms of melanoma with *in vivo* investigations on pathology and immunology. The D4M-3A cell line is a clinically relevant model for both *in vitro* and *in vivo* studies of metastatic melanoma.

Source

The D4M-3A cell line was derived from a male transgenic Braf/Pten mouse.[2]

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, human, 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

D4M-3A mouse melanoma 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

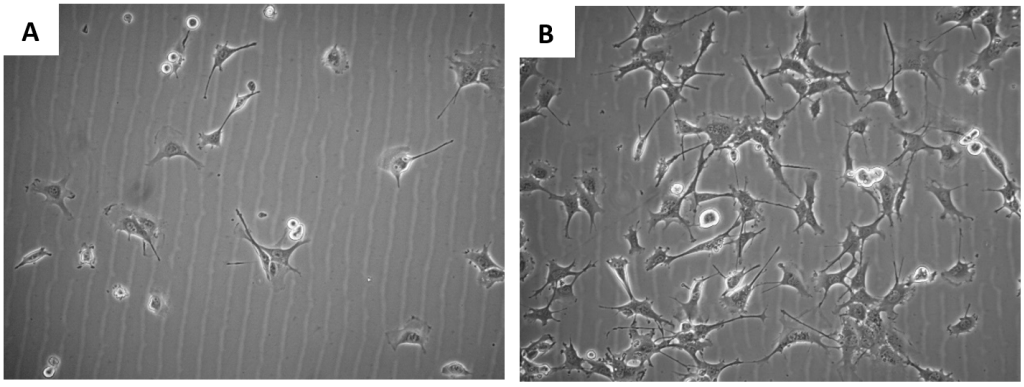

Figure 1. Brightfield images of D4M-3A cells one (**A**) and two (**B**) days after thaw in a T225 flask.

Protocols

Thawing Cells

D4M-3A cells proliferate rapidly. We recommend thawing cells into a T225 flask.

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 D4M-3A Expansion Medium comprised of DMEM/F12 Plus (Cat. No. SCM162) with 5% Heat-Inactivated FBS (Cat. No. ES-009-B) and 1X penicillin/streptomycin (Cat. No. TMS-AB2-C, optional).
2. Remove the vial of frozen D4M-3A 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 D4M-3A 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 50 mL of D4M-3A Expansion Medium.
10. Transfer the cell mixture to a T225 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. D4M-3A 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 T225 tissue culture flask containing the 80% confluent layer of D4M-3A cells.
3. Rinse the flask with 50 mL 1X PBS. Aspirate after the rinse.

4. Apply 25 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 25 mL of D4M-3A Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 50 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 D4M-3A Expansion Medium to the conical tube and resuspend the cells thoroughly.
    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

D4M-3A mouse melanoma cells may be frozen in D4M-3A Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. CA Cancer J Clin 2004, 54(3): 131-149.
2. Pigmnt Cell Melanoma Res 2014, 27(3): 495-501.

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