Melan-a Mouse Melanocyte Cell Line

Immortalized Cell Line

Cat. # SCC202

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.

Data Sheet

Background:

Melanoma is one of the most malignant skin cancers, being responsible for three out of four skin cancer-related deaths[1]. The increasing worldwide incidence of melanoma has spurred intensive research into the molecular mechanisms that give rise to malignant forms. Melanoma arises from melanocytes, pigment-producing cells that are found throughout the body. The availability of robust cellular models that reflect normal skin melanocytes is essential to understanding the etiology and abnormalities of melanomas.

The Melan-a cell line is an immortal melanocyte cell line derived from embryonic mouse skin and was the first established non-tumorigenic mouse melanocyte cell line[2]. Melan-a cells are diploid in chromosome number and syngeneic with C57BL mice and sublines of B16 melanoma, enabling comparative studies. Melan-a cells exhibit the pigmentation and morphology of normal melanocytes and express gp100 as well as the melanocyte marker MelanA. Melan-a cells proliferate in the presence of the tumor promoter tetradecanoyl phorbol acetate (TPA). The Melan-a cell line is a well-established model for melanocyte biology.

Source

The Melan-a cell line was derived from trunk skin of embryonic day 18 C57BL mice[2].

Storage and Handling

Melan-a Mouse Melanocyte 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

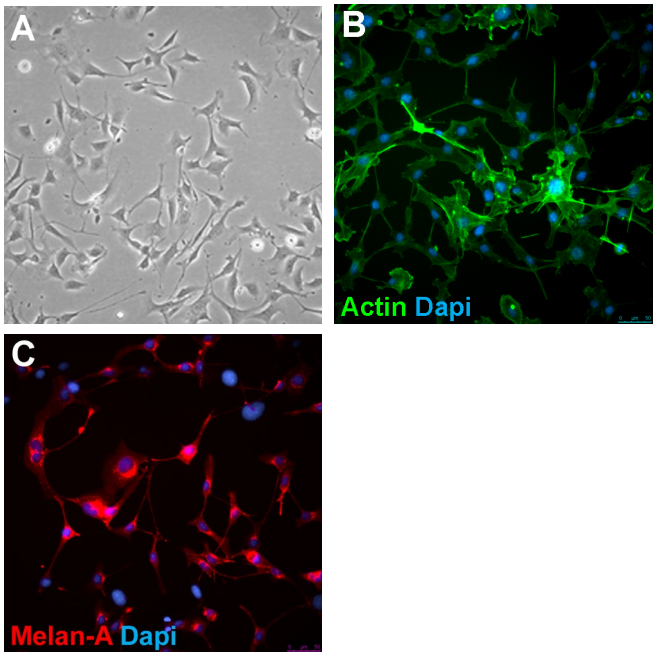

Figure 1. Bright-field image of cells, 2 days after thaw (**A**). Cells express actin (**B**, Sigma P5282) and MelanA (**C**).

References

1. *Nat Rev Dis Primers.* 2015; 1:15003.
2. *In J Cancer.* 1987; 39(3):414-418.

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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.

   Melan-a Expansion Medium: cells are thawed and expanded in RPMI-1640 (Sigma Cat. No. R8758), 10% FBS (Cat. No. ES-009-B), and 200 nM phorbol-12-myristate-13-acetate (TPA) (Cat. No. 524400).
2. Remove the vial of frozen Melan-a 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 Melan-a 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 Melan-a 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 Cells

Note: Melan-a cells proliferate relatively slow. It may take several days before flask is 80-85% confluence and ready to be passage.

1. Do not allow the cells to grow to confluency. Melan-a cells should be passaged at ~80-85% confluence.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of Melan-a cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 mL of Accutase and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 5-7 mL of Melan-a Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of Melan-a Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density. Typical split ratio is 1:4 or 1:6.

Cryopreservation of Cells

Melan-a Mouse Melanocyte Cell Line may be frozen in RPMI-1640 medium with 10%FBS and 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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