Data Sheet

SK-MEL-147 Human Melanoma Cell Line

Cancer Cell Line

SCC440

Pack Size: ≥ 1x10^6

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Melanoma is considered the most dangerous form of skin cancer. It develops in melanocytes, the pigment producing cells of the skin. Melanoma accounts for a very small number of all skin cancers. Even though it accounts for only about 1% of total diagnosed skin cancer cases, melanoma is responsible for the largest number of skin cancer deaths.[2] Melanoma incidence rates have increased over time, although mortality rates from melanoma have improved over the last decade due to advances in treatment.

SK-MEL-147 is a human metastatic melanoma cell line. It retains a Q61R NRAS point mutation which is associated with a more aggressive phenotype, higher metastasis risk, and poorer prognosis.[1] While there has been an improvement in understanding BRAF-mutant melanoma and its therapeutic strategies, solutions to NRAS-mutant melanoma have been limited. Further development of immune therapies for NRAS-specific mutants remains highly relevant. The SK-MEL-147 cell line expresses PMEL and MelanA proteins, two of the most common melanoma markers.

Source

The SK-MEL-147 was derived from an unspecified metastatic site in a patient with malignant melanoma. This cell line was developed by the LJ Old lab at Memorial Sloan Kettering Cancer Center.

Short Tandem Repeat

D3S1358: 17, 18	D7S820: 9, 11	vWA: 17, 18	FGA: 21, 24	D8S1179: 12, 17
D21S11: 30, 31	D18S51: 13, 17	D5S818: 10, 13	D13S317: 9, 11	D16S539: 12, 13
TH01: 6, 9.3	TPOX: 8, 12	CSF1PO: 10, 12	AMEL: X	Penta D: 9, 12
Penta E: 12, 13	Mouse: NA			

Quality Control Testing

- Cells are verified to be of human origin and negative for inter-species contamination from mouse, rat, chinese hamster, Golden Syrian hamster, and non-human primate (NHP) as assessed using a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases using a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

The SK-MEL-147 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

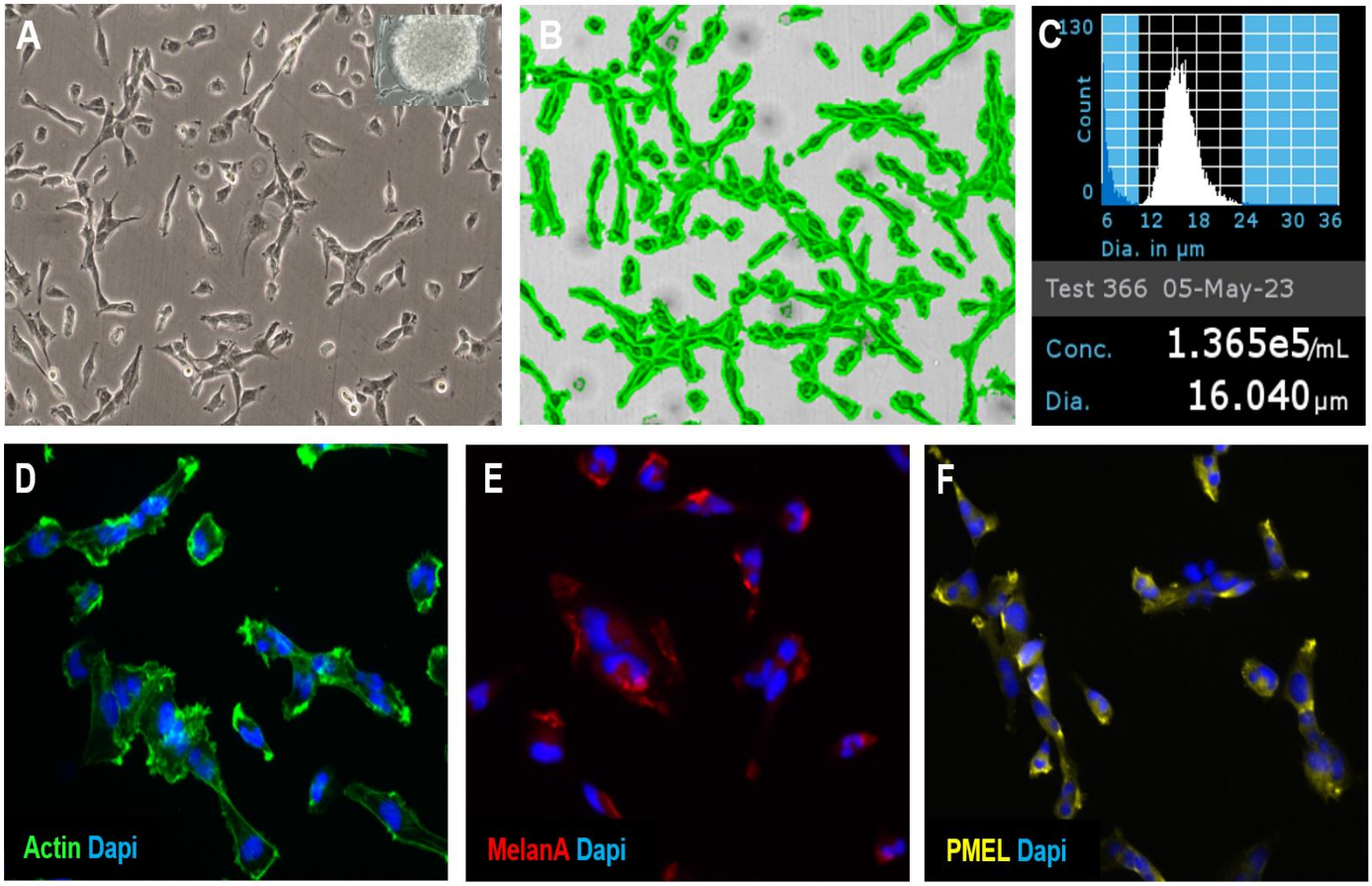

Figure 1. **A**. Brightfield image of SK-MEL-147 cells one day after thaw in a T75 flask.
Note: As cells grow in culture, spheroids may form and detach (A, inset).
B. Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000).
C. Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter (PHCC360KIT) using 60 µm sensors. **D**. Cells express actin (49409), **E**. MelanA (Thermo Scientific PIPA5101023), and **F**. PMEL (Thermo Scientific PIPA599174).

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 SK-MEL-147 Expansion Medium comprising RPMI1640 (R8758) containing 10% FBS (ES-009-B), and 2 mM L-Glutamine (TMS-002-C).
2. Remove the vial of frozen SK-MEL-147 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 SK-MEL-147 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 SK-MEL-147 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

Cells are loosely adherent. Some spheroids may form in the culture.

1. Do not allow the cells to grow to confluency. SK-MEL-147 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 SK-MEL-147 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 SK-MEL-147 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 SK-MEL-147 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

SK-MEL-147 cells may be frozen in SK-MEL-147 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Muñoz-Couselo E, Adelantado EZ, Ortiz C, García JS, Perez-Garcia J. 2017. NRAS-mutant melanoma: current challenges and future prospect. Onco Targets Ther. 10:3941-3947.
2. Schadendorf D, Fisher DE, Garbe C, Gershenwald JE, Grob J-J, Halpern A, Herlyn M, Marchetti MA, McArthur G, Ribas A, et al. 2015. Melanoma. Nat Rev Dis Primers. 1:15003.
3. Zhang S, Chen K, Liu H, Jing C, Zhang X, Qu C, Yu S. 2021. PMEL as a prognostic biomarker and negatively associated with immune infiltration in skin cutaneous melanoma (SKCM). J Immunother. 44(6):214-223.
4. Kaufmann WK, Nevis KR, Qu P, Ibrahim JG, Zhou T, Zhou Y, Simpson DA, Helms-Deaton J, Cordeiro-Stone M, Moore DT, et al. 2008. Defective cell cycle checkpoint functions in melanoma are associated with altered patterns of gene expression. J Invest Dermatol. 128(1):175-187.
5. Houghton AN, Brooks H, Cote RJ, Taormina MC, Oettgen HF, Old LJ. 1983. Detection of cell surface and intracellular antigens by human monoclonal antibodies. J Exp Med. 158(1): 53-65.

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