# DM6 Human Melanoma Cell Line

Cancer Cell Line

**Cat. # SCC221**

**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

Although cases of malignant melanoma show a high survival rate when treated early, patients with later stages of the disease and chemotherapy-resistant forms have a poor prognosis.[1] Relevant cellular models of human chemoresistant melanoma are of great importance for understanding mechanisms of resistance and in aiding the development of effective new treatments, especially targeted immunotherapies.

The DM6 human melanoma cell line is an established model for immunotherapy and chemoresistance. DM6 human melanoma cells harbor the melanoma driver mutation *BRAF* V600E and express the melanoma markers gp100 and MelanA.[2] DM6 human melanoma cells express HLA-A2 antigen and are competent to induce T-cell cytotoxic response.[3] The antigenic expression profile of this cell line has contributed to its utilization as a viable model for evaluating targeted cancer immunotherapy.[4] DM6 melanoma cells are extremely resistant to the common first-line chemotherapy for malignant melanoma.[5] The unique features of the DM6 human melanoma cell line make it a system with high clinical relevance.

## Source

The DM6 human melanoma cell line was derived from surgically excised lymph nodes dispersed into single cell suspensions.[2]

## Short tandem repeat (STR) Profile

| | |
|---|---|
| D3S1358: 16, 18 | D16S539: 10, 11 |
| TH01: 9 | CSF1PO: 8, 11 |
| D21S11: 29, 30 | Penta D: 10, 12 |
| D18S51: 12 | vWA: 17, 19 |
| Penta E: 10, 11 | D8S1179: 14 |
| D5S818: 12, 13 | TPOX: 9, 11 |
| D13S317: 13 | FGA: 24, 27, 28 |
| D7S820: 7, 11 | Amelogenin: X |

Cancer cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

## Storage & Handling

DM6 human 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 Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

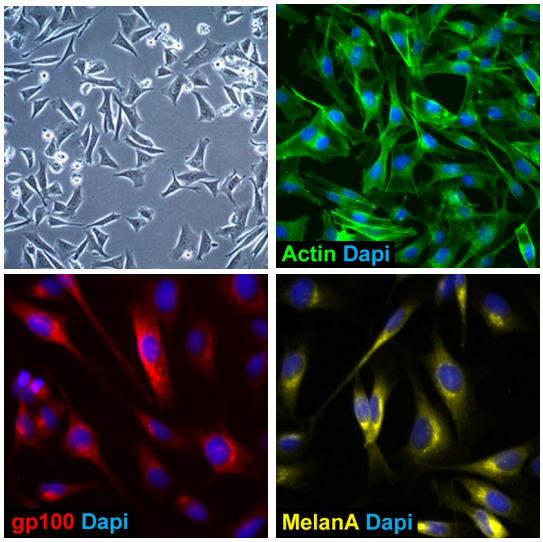


**Figure 1.** DM6 cells one (**A**) day after thawing in a T75 flask. Cells express actin (**B**, Phalloidin-FITC; Sigma P5282), gp100 (**C**), and MelanA (**D**).

## References

1. *Clin Cancer Res.* 2006; 12(7 Pt 2): 2312s-2319s.
2. *Cancer Immunol Immunother.* 2000; 48(12): 661-672.
3. *Cancer Res.* 1990; 50(3): 492-498.
4. *Sci Transl Med.* 2017; 9(408): eaan4220
5. *Clin Cancer Res.* 2009; 15(2): 502-510.

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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.

   DM6 Expansion Medium: Cells are thawed and expanded in DMEM Complete (Cat. No. SLM-241-B) which contains DMEM high glucose (Sigma Cat. No. D6546) supplemented with 2 mM L-Glutamine (Cat. No. TMS-002-C) and 10% FBS (Cat. No. ES-009-B).
2. Remove the vial of frozen DM6 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 DM6 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 DM6 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

1. Do not allow the cells to grow to confluency. DM6 cells should be passaged at ~80-85% confluence.
2. Carefully remove the medium from the T75 tissue culture flask containing the DM6 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 DM6 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 DM6 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 to 1:6.

### Cryopreservation of Cells

DM6 human melanoma cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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