

**B16 Cells | 305154****General information****Description**

The B16 cell line is a widely used murine model derived from melanoma tumors in C57BL/6 mice. This line is extensively employed in research due to its ability to form melanotic tumors that closely resemble human melanoma in terms of growth characteristics and metastatic potential. The cell line exists in various subtypes, such as B16-F0, B16-F1, and B16-F10, with each subtype demonstrating varying degrees of metastatic capability; for instance, B16-F10 is highly metastatic compared to B16-F0. These variations allow researchers to select an appropriate model based on the specific requirements of their studies concerning tumor aggressiveness and metastasis.

B16 cells are instrumental in understanding the molecular and cellular mechanisms of melanoma progression and testing anti-cancer therapies. Their melanin-producing ability makes them particularly useful for studies on melanogenesis and its regulation. Furthermore, the B16 cell line serves as an essential tool for vaccine development and immunotherapy experiments, offering insights into tumor-immune system interactions and the efficacy of immunomodulatory agents. The adaptability of these cells to various in vivo and in vitro environments underscores their significance in translational and preclinical research aimed at melanoma treatment and prevention.

**Organism** Mouse**Tissue** Skin**Disease** Mouse melanoma**Synonyms** B-16, B16 melanoma, B16 subline B78, B78**Characteristics****Breed/Subspecies** C57BL/6**Gender** Male**Morphology** Mixture of spindle-shaped and epithelial-like cells**Growth properties** Adherent**Regulatory Data****Citation** B16 (Cytion catalog number 305154)**Biosafety level** 1

**B16 Cells | 305154****NCBI\_TaxID** 10090**CellosaurusAccession** CVCL\_F936**Biomolecular Data****Tumorigenic** Yes**Products** Melanin**Handling****Culture Medium** EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO<sub>3</sub>, w: EBSS (Cytion article number 820100a)**Supplements** Supplement the medium with 10% FBS and 1% NEAA**Dissociation Reagent** Accutase**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.**Fluid renewal** 2 to 3 times per week**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Flask Coating

None

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.