cyagen | OriCell

User Manual

OriCell™ B16-F10 Mouse Skin Melanoma Cell Line

Catalog No. M5-0801

We help you discover life

Introduction

The B16-F10 mouse skin melanoma cell line was originally isolated in 1954 by researchers at The Jackson Laboratory from skin melanoma tissue of C57BL/6J mice. The cells exhibit a mixed spindle-shaped and epithelial-like morphology. The laboratory maintained B16 cells through continuous in vivo passage, and the B16-F10 subline was established from lung metastatic melanoma tissue obtained after the 10th consecutive passage of the parental B16-F0 cell line.

OriCell™ B16-F10 Mouse Skin Melanoma Cell Line is widely used for tumor model establishment and studies of tumor mechanisms, particularly in the fields of melanoma research and tumor immunology.

Note: **This product is intended for research use only and is not for diagnostic, therapeutic, clinical, household, or any other applications.**

When citing our products in academic publications, please use the following format: "OriCell™ [Product Name] + [Catalog Number], from Cyagen Biosciences."

Product Information

Product Name	OriCell™ B16-F10 Mouse Skin Melanoma Cell Line
Alternative Name	B16-F10
Catalog Number	M5-0801
Amount of Cells	1×10^6 cells/vial
Tissue Origin	Mouse Skin
Cell Characteristics	Adherent Growth; Mixed spindle-shaped and epithelial-like morphology
Culture Conditions	95% air; 5% CO_2; 37 °C
Culture Medium	RPMI-1640 + 10% FBS
Doubling Time	48 ~ 72 h
Biosafety Level	1
Storage at	Liquid Nitrogen (-196 °C)
Precautions	To promote melanin production, use DMEM + 10% FBS.

Note: This product is manufactured under strict aseptic conditions. You may choose to add antibiotics during subsequent culturing based on your specific needs.

The Morphology of OriCell™ B16-F10 Mouse Skin Melanoma Cell Line

Validation of Tumorigenicity Data

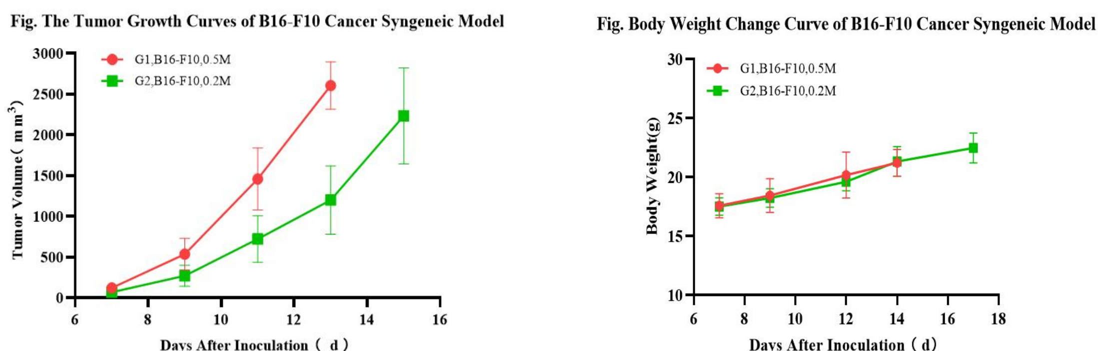

Figure 1. Subcutaneous Tumor Growth and Body Weight Curves (n = 8).

B16-F10 cells were subcutaneously inoculated into 7-week-old C57BL/6J mice, and tumor volumes were measured at different time points. Inoculation doses were 5×10^5 and 2×10^5 cells per mouse, with data presented as Mean ± SEM. The results indicate that B16-F10 cells readily establish tumors in C57BL/6J mice. Tumor volume is expected to reach 100 – 200 mm^3 by day 7 post-inoculation and reach the experimental endpoint of 2000 mm^3 on day 15. The projected therapeutic window is approximately 8 days.

QC

- Pass the detection of bacteria, fungi, mycoplasma, and endotoxins.
- Verified by cell recovery viability testing.
- Verified by STR analysis.

Please refer to "COA" for details.

General Handling Principles

1. Maintain strict aseptic technique. Ensure complete sterility throughout all procedures, particularly within the laminar flow hood and incubator.
2. Follow standardized protocols. Adhere strictly to the product manual. Implement rigorous control over experimental variables and include appropriate parallel controls.
3. Use high-quality consumables and reagents. This product requires culture vessels suitable for adherent growth, and the reuse of these vessels is not recommended. The reagents used must be validated for reliability, cell compatibility, and batch-to-batch consistency.

Note: **The cryopreservation medium of this product contains DMSO, which may pose potential risks. Please handle it with care.**

Abbreviation	Name	Cat. No.
FBS	Fetal Bovine Serum	See official website
BCS	Bovine Calf Serum	SBCST-01001
Glu	Glutamine	SGLU-10201
SP	Sodium Pyruvate	SCSP-10301
Dex	Dexamethasone	SDEX-10401
NBCS	Newborn Calf Serum	NCSST-01001
HS	Horse Serum	SCHST-01001
NEAA	Non Essential Amino Acid	NEAA-10201
β-mer	β-mercaptoethanol	BMER-10301
P/S	Penicillin- Streptomycin	ATPS-10001
ITS	Insulin、Transferrin、Selenite	ITSS-10201

Thawing and Culturing of Cells

Materials Required

- OriCell™ B16-F10 Mouse Skin Melanoma Cell Line (Cat. No.: M5-0801)
- OriCell™ Complete Medium For B16-F10 Cell Line (Cat. No.: CMM5-0801)

Steps

Note: **If thawing is planned within 24 hours of receipt, store the cells in an ultra-low temperature freezer at -80 °C. For long-term storage (>24 hours) , keep them in liquid nitrogen. Before thawing, transfer the cells from liquid nitrogen to -80 °C and hold them there for 10 minutes. This will allow any residual liquid nitrogen to evaporate and prevent vial explosion.**

1. Pre-warm the water bath to 37 °C.
2. Warm the complete medium to 37 °C.
3. Add at least 5 mL of pre-warmed complete medium to a 15 mL centrifuge tube for subsequent use.
4. Remove the cryovial containing cells from the −80 °C freezer, immerse it in the 37 °C water bath, and gently and quickly swirl to thaw the cryopreservation medium.

   Note:

   (1) Gently swirl the cryovial during thawing to ensure rapid and uniform thawing.

   (2) Avoid submerging the cap in water to prevent contamination.

   (3) Stop thawing in the water bath when only a single ice crystal (approximately 2 mm in diameter) remains, then continue gently swirling the vial until it is completely thawed.

5. Wipe the outer surface of the cryovial with 75% ethanol.
6. In a biosafety cabinet, open the cryovial and transfer the cell suspension to the prepared

centrifuge tube using a Pasteur pipette.

7. Rinse the cryovial once with 1 mL of complete medium to collect residual cells and minimize loss.
8. Centrifuge the cell suspension at 250 × g for 4 minutes.

   Note: **Please calculate the corresponding rotational speed using the formula: $RCF = 1.118 \times 10^{-5} \times r \times RPM^2$ (where RCF is the relative centrifugal force, r is the rotor radius in cm, and RPM is the rotational speed).**

9. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium, gently resuspend the cell pellet by pipetting up and down to mix thoroughly.
10. Seed the cells into a T25 flask or culture vessel with an equivalent growth surface area. Add sufficient complete medium so that the total volume in a T25 flask is no less than 5 mL.
11. Gently swirl the flask to evenly distribute the cells, then incubate in a CO_2 incubator at 37 °C with 5% CO_2 and saturated humidity.

   Note: **Do not move or observe the cells within the first 2 hours after seeding, as this may impair cell adhesion, causing poor morphology, clumping, and uneven attachment.**

12. On the day after recovery, observe cell status and either replace the medium with fresh complete medium or passage the cells as needed.

   Note: **If an excessive number of floating cells or any abnormal conditions are observed, investigate promptly and contact us for assistance.**

13. Replace the complete medium every 3 days until the cells reach approximately 95% confluence, at which point they are ready for passage.

Passaging of Cells

Materials Required

- OriCell™ 0.25% Trypsin-0.04% EDTA Solution (Cat. No.: TEDTA-10001)
- OriCell™ Phosphate-Buffered Saline Solution (1X) (Cat. No.: PBS-10001)
- OriCell™ Complete Medium For B16-F10 Cell Line (Cat. No.: CMM5-0801)

Steps

1. Pre-warm the complete medium, PBS and trypsin to 37 °C.
2. Remove the medium in the culture vessel.
3. Gently wash the cells twice with PBS (approximately 3 mL for a T25 flask or 6 mL for a T75 flask). Ensure thorough washing but avoid excessive force. Then remove the PBS.
4. Add trypsin (approximately 1.5 mL for a T25 flask or 3 mL for a T75 flask), quickly spread it to ensure full coverage of the cell layer.
5. Observe the cells under a microscope. When approximately 70% to 80% of the cells have shrunk and become round, gently tap the outer wall of the culture vessel to detach the cells from the surface.
6. Immediately add complete medium (approximately 3 mL for a T25 flask or 6 mL for a T75 flask), then gently swirl the culture vessel to mix the medium and trypsin, stopping the digestion process.
7. Collect the cell suspension using a pipette, gently pipetting along the bottom of the vessel several times to ensure maximal recovery of the cells.

   Note: **Pipetting should be performed gently to avoid creating excessive bubbles, as this may cause cell damage or loss.**

8. Transfer the cell suspension to a centrifuge tube. Rinse the culture vessel once with PBS (approximately 3 mL for a T25 flask or 6 mL for a T75 flask) to collect residual cells and add

the wash to the centrifuge tube.

9. Centrifuge all collected cell suspensions at 250 × g for 4 minutes.
10. Carefully remove the supernatant after centrifugation. Add 2 mL of complete medium and gently resuspend the cell pellet by pipetting up and down to thoroughly mix.
11. Seed the cells into a suitable culture vessel at a density of (2–3) × 10^4 viable cells/cm^2, or adjust the seeding density based on the actual growth conditions of the cells.

    Note: **We recommend manual cell counting when conditions permit and counting efficiency is high, in order to obtain an accurate cell concentration to guide seeding. If precise counting is not feasible, subculturing at an appropriate ratio is a better alternative. Typically, B16-F10 cells are passaged at a ratio of 1:4 to 1:6, with cells reaching passage confluence within 72 hours. Please adjust the subculture ratio according to the actual condition of the cells.**

12. Gently agitate the vessel to ensure uniform cell distribution and place it in an incubator at 37 °C, 5% CO_2, and saturated humidity.
13. On the day after passaging, observe the cell condition. If a significant number of floating cells are present, replace the culture medium.
14. Replace the culture medium every 3 days. When cells reach 95% confluence, passage or cryopreserve the cells.

Cryopreservation of Cells

Materials Required

- OriCell™ NCR Protein-Free Cryopreservation Medium For General Use (Cat. No.: NCPF-10001)
- OriCell™ NCR Cryopreservation Medium For General Use (Cat. No.: NCRC-10001)

Steps

1. Cells should be cryopreserved once they reach an appropriate density suitable for passaging.
2. For cell digestion, please refer to passaging steps 1-9 above.
3. Carefully remove the supernatant after centrifugation and gently resuspend the cells in an appropriate volume of cryopreservation medium.
4. Aliquot the cells into cryovials according to the desired cell number or proportion.

   Note: **If accurate cell counting is not feasible, we recommend aliquoting cells proportionally for freezing. Prolonged exposure to non-culture conditions will significantly compromise cell viability. Maintain the cells at 4 °C during counting to minimize metabolic activity and preserve cell integrity.**

5. When using any of the recommended NCR cryopreservation media above, cryovials can be directly placed individually into a -80 °C freezer.

   Note: **Avoid opening the freezer door during the first 4 hours of freezing, as temperature fluctuations can adversely affect cell viability.**

6. After approximately 8 hours, transfer the cryovials to liquid nitrogen for long-term storage.

   Note: **Do not store the cryovials at -80°C for more than 48 hours.**

Cyagen Biosciences (Suzhou) Inc. reserves all rights to the technical documents of OriCell™ cell culture products. Without the written permission of Cyagen Biosciences (Suzhou) Inc. any part of this document shall not be adapted or reprinted for other commercial purposes.