Data Sheet

Assay Ready MB49 Mouse Bladder Carcinoma Cell Line

SCCAR10148–1VL

Pack Size: 1 vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

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

Background

The mouse urothelial carcinoma cell line, MB49 is widely used as an *in vitro* and *in vivo* model of bladder cancer. MB49 cells are derived from C57BL/Icrf-a' mouse bladder epithelial cells that were transformed by a single 24-hour treatment with the chemical carcinogen 7, 12-dimethylbenz[a]anthracene (DMBA) on the second day of a long term primary culture.[1] Transformed cells transplanted into syngeneic mice were shown to generate carcinomas.[1] While of male origin, karyotype analyses indicate the loss of the Y chromosome in 100% of the cells analyzed.[2] This abnormality is a frequent early event in human bladder cancer. A recent study indicates that MB49 cells recapitulate key features of sex differences in bladder tumor growth.[3] MB49 implantation in mice resulted in significantly larger tumors in males than females. In the presence of dihydrotestosterone, MB49 cells exhibited enhanced proliferation in a dose-dependent manner. In contrast, MB49 cells were unresponsive to the pregnancy hormone, human chorionic gonadotrophin (hCG).[3] MB49 cells exhibit low to no expression of MHC-Class I and II molecules.[4] However, upon exposure to IFN-γ, the expression of MHC Class I and II are significantly upregulated.[4]

MB49 cells proliferate rapidly and do not form a 100% confluent monolayer. At ~70% confluence, the cells will tend to detach in small clumps that float in the medium. About 10-20% of the cells will be attached with a spindle-like epithelial morphology, while the remainder will appear rounded.

Source

MB49 cells are derived from C57BL/Icrf-a' mouse bladder epithelial cells that were transformed by a single 24-hour treatment with the chemical carcinogen 7, 12-dimethylbenz[a]anthracene (DMBA).

Short Tandem Repeat

M18-3: 16	M4-2: 20.3	M6-7: 15	M19-2: 14	M1-2: 19
M7-1: 26.2	M1-1: 15, 16	M3-2: 13, 14	M8-1: 16	M2-1: 16
M15-3: 22.3	M6-4: 18	M11-2: 16	M17-2: 14, 15	M12-1: 17
M5-5: 17	MX-1: 28, 29	M13-1: 17	D8S1106: NA	D4S2408: NA

Quality Control Testing

- The Assay ready MB49 cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

The assay ready MB49 cells should be stored in liquid nitrogen until use.

Do not expand, passage, or cryopreserve these cells after recovery.

Representative Data

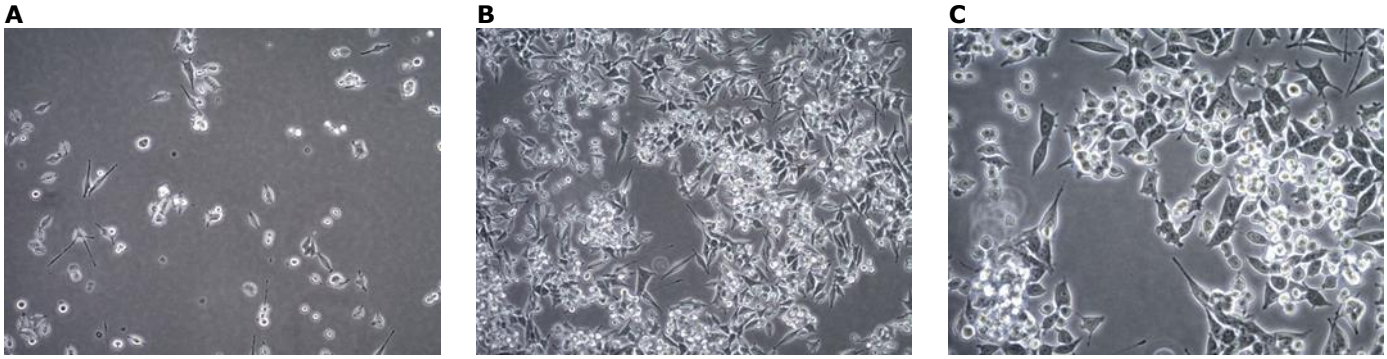

Figure 1. Bright-field image of MB49 cells after thaw in a T175 flask. (**A**) Day 1, (**B and C**) Day 3. Higher magnification (**C**) image illustrates the presence of loosely attached spheroids in culture.

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal T175 tissue cultureware flask without any additional coating.

   Cells are thawed and can be cultured in DMEM Complete Medium (SLM-241-B) or in DMEM-High Glucose (D5796) with 10% FBS (ES-009-B) and 1X Penicillin/Streptomycin (optional, TMS-AB2-C).
2. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
3. 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.
4. Using a 10 mL pipette, slowly add dropwise 9 mL of MB49 Expansion Medium (Step 2 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.
5. 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.
6. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
7. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
8. Resuspend the cells in 35-40 mL of MB49 Expansion Medium.

9. Transfer the cell mixture to a T175 tissue culture flask.
10. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.
11. The next day, exchange the medium with 30-45 mL of fresh MB49 Expansion Medium.
12. At day 3 total cell number should be 10 million viable cells.
13. Rinse the flask with 20 mL 1X PBS. Aspirate after the rinse.
14. Apply 8-10 mL of Accutase® (SCR005) and incubate in a 37 °C incubator for 3-5 minutes.
15. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
16. Add 10-15 mL of MB49 Expansion Medium to the flask.
17. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 50 mL conical tube.
18. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
19. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
20. Apply 5-8 mL of MB49 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.
21. Count the number of cells using a hemocytometer or a Scepter™ 3.0 handheld automated cell counter.

References

1. Effects of Donor Age on Neoplastic Transformation of Adult Mouse Bladder Epithelium *in vitro*. 1979 Apr. JNCI: Journal of the National Cancer Institute.
2. Fabris VT, Lodillinsky C, Pampena MB, Belgorosky D, Lanari C, Eiján AM. 2012. Cytogenetic characterization of the murine bladder cancer model MB49 and the derived invasive line MB49-I. Cancer Genetics. 205(4):168–176.
3. White-Gilbertson S, Davis M, Voelkel-Johnson C, Kasman LM. 2016. Sex differences in the MB49 syngeneic, murine model of bladder cancer. Bladder. 3(1):22.
4. Lattime EC, Gomella LG, McCue PA. 1992. Murine bladder carcinoma cells present antigen to BCG-specific CD4+ T-cells. Cancer Research. 52(15):4286–4290.

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