MB49 Murine Bladder Carcinoma Cell Line

Cancer Cell Line

Cat. # SCC148

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.

Certificate of Analysis

Background

MB49, a mouse urothelial carcinoma cell line 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 expressions 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.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for inter-species contamination from rat, chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination

Storage and Handling

MB49 Murine Bladder Carcinoma cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

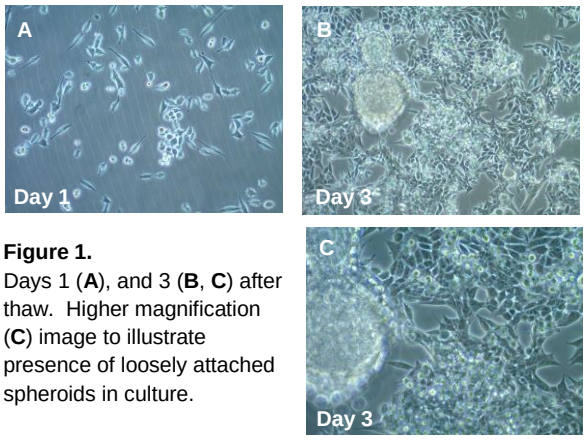

Figure 1.
Days 1 (**A**), and 3 (**B**, **C**) after thaw. Higher magnification (**C**) image to illustrate presence of loosely attached spheroids in culture.

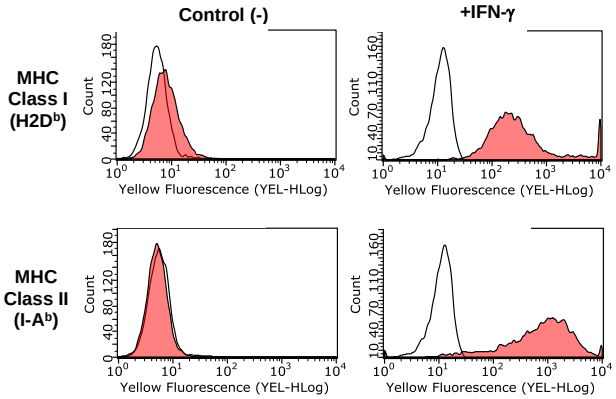

Figure 2. Induced expression of MHC Class I and II molecules by treatment with IFN-γ. In vitro cultured MB49 cells were cultured for 2 days in the absence (Control) or presence (+IFN-γ) of 500 IU/mL of recombinant mouse interferon-γ (IFN-γ). Cells were blocked with 10 μg/mL of 2.4G2 monoclonal antibody and stained with R-phycoerythrin (PE) labeled anti-H-2Db (MHC Class I) and anti-I-Ab (MHC Class II) antibodies for flow analyses.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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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.

   Cells are thawed and can be expanded in DMEM Complete Medium (Cat. No. SLM-241-B) **or** in DMEM-High Glucose (Sigma Cat. No. D5796) with 10% FBS (Cat. No. ES-009-B) and 1X Penicillin/Streptomycin (optional; Cat. No. TMS-AB2-C).
2. Remove the vial of frozen MB49 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 MB49 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 10-15 mL of MB49 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.
12. The next day, exchange the medium with 10-15 mL of fresh MB49 Expansion Medium.
13. **Media exchanges:** Collect any floating cells. Centrifuge, aspirate and resuspend floaters in 2 mL fresh media and add back to the flask containing 10 mL fresh media. Exchange with fresh medium every two to three days.
13. When the cells are approximately 80-85% confluent, they can be dissociated with Accutase (Cat. No. SCR005) or trypsin-EDTA (Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use.

Subculturing Cells

1. Collection of floating MB49 cell clumps: Carefully collect the medium containing floating clumps of MB49 cells from the confluent T75 tissue culture flask. Centrifuge to collect the cell clumps. Aspirate and resuspend in 2 mL of MB49 expansion medium and set aside.
2. Detachment of adherent MB49 cells: Rinse the T75 flask twice with 10 mL 1X PBS. Aspirate after each rinse.
3. Apply 3-5 mL of Accutase or trypsin-EDTA solution 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 8 mL of MB49 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 mL of the floating cell suspension set aside in step 1 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 1:6 – 1:10).

Cryopreservation of Cells

MB49 Murine Bladder Carcinoma Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Summerhayes IC, Franks LM (1979) Effects of donor age on neoplastic transformation of adult mouse bladder epithelium in vitro. *J Natl Cancer Inst* 62(4): 1017 - 1023.
2. Fabris VT, Lodilinsky 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 Genet* 205(4): 168 - 76.
3. White-Gilbertson S, Davis M, Voelkel-Johnson C, Kasman LM (2016) Sex differences in the MB49 syngeneic, murine model of bladder cancer. *Bladder (San Franc)* 3(1): PMID: 26998503.
4. Lattime EC, Gomelia LG, McCue PA (1992) Murine bladder carcinoma cells present antigen to BCG-specific CD4+ T-cells. Cancer Res 52(15): 4286 - 90.

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