# MB49 Murine Bladder Carcinoma Cell Line culture protocol - Cat # 153368

## Materials required:

| Material |
|---|
| MB49 Murine Bladder Carcinoma Cell Line (CancerTools **Cat. No. 153368**) |
| MB49 growth medium:<br>DMEM Complete Medium or DMEM-High Glucose Medium<br>10% Foetal Bovine Serum (FBS)<br>1x Penicillin/Streptomycin (Optional) |
| Cryopreservation medium:<br>Growth medium<br>10% DMSO |
| Phosphate buffered saline (PBS) |
| Accutase or trypsin-EDTA solution |

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## Thawing cells:

***Note: The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.***

1. Prepare MB49 growth medium: DMEM Complete Medium or DMEM-High Glucose Medium with 10% FBS and 1X Penicillin/Streptomycin (optional), to thaw and expand the cells. The cells can grow on normal tissue culture ware surfaces without any additional coating.
2. Remove the vial of frozen MB49 cells from liquid nitrogen and thaw rapidly by incubating 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 microbiological safety cabinet, use a 1- or 2 mL pipette to transfer the cells into 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 growth medium (Step 1 above) into 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.**
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 growth medium.
10. Transfer the cell mixture into 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 growth medium.
13. **Media exchanges:** Collect any floating cells. Centrifuge, aspirate and resuspend floaters in 2 mL of fresh media and add back into the flask containing 10 mL of fresh media. Exchange with fresh medium every 2-3 days.
14. When the cells are approximately 80-85% confluent, they can be dissociated with Accutase or trypsin-EDTA solution and further passaged or frozen for later use.

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## Subculturing cells:

***Note: 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. Cells adhere slowly at passage 3 and onwards.***

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 growth medium and set aside.
2. **Detachment of adherent MB49 cells:** Rinse the T75 flask twice with 10 mL of 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 growth medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells into 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. Split and plate the cells into new flasks at the desired density (typical split ratio is 1:6 – 1:10).

## Cryopreservation of cells:

1. MB49 cells may be cryopreserved/frozen in the MB49 growth medium with 10% DMSO using a Nalgene slow freeze Mr. Frosty container.
2. Transfer MB49 cells into liquid nitrogen for long-term storage.

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