MCA205 Murine Fibrosarcoma Cell Line

Tumor Cell Line

Cat. # SCC173

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.

Data Sheet

Background:

Cancer immunotherapy is a rapidly expanding field that has produced significant advances in cancer treatment. Understanding the interactions of tumor cells with the immune system is crucial to the development of effective immunotherapeutic approaches. Fibrosarcoma is a rare yet aggressive soft-tissue sarcoma characterized by frequent tumor recurrences and a high degree of resistance to radio- and chemotherapy.[1] MCA-205 is a weakly immunogenic fibrosarcoma-derived cell line that has been used in many studies to induce tumor-reactive T lymphocytes, the key aspect of adoptive immunotherapy.[2] MCA-205 cells are widely utilized as stimulators for inducing cytokine expression.[3] The MCA-205 mouse fibrosarcoma cell line is an excellent model for studying the immune responses to tumor cells and for supporting the development of targeted cancer immunotherapies.

Source

Non-GMO. MCA-205 was derived from 3-methylcholanthrene-induced fibrosarcoma in C57BL/6 mice. Tumors were maintained in vivo by serial subcutaneous transplantation in syngeneic mice and single-cell suspensions were prepared from solid tumors by enzymatic digestion.[4] From these cells the MCA-205 cell line was established and maintained in vitro.

Storage and Handling

MCA-205 Murine Fibrosarcoma Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

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.

Representative Data

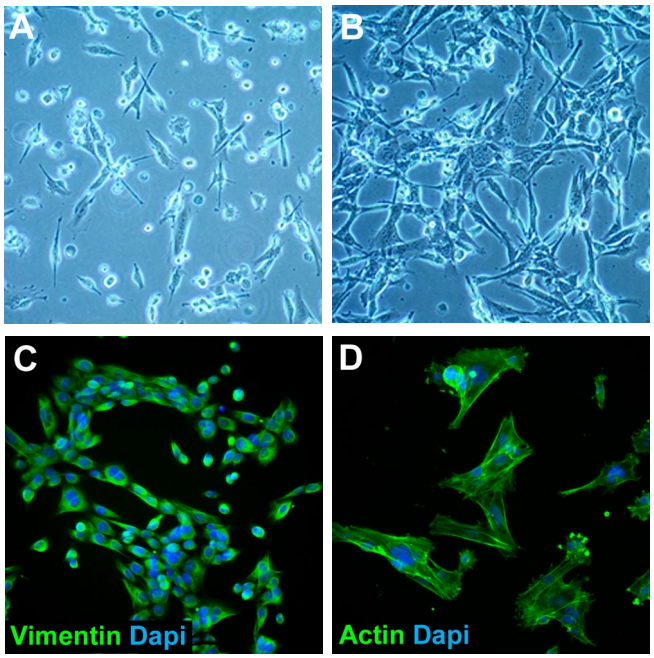

Figure 1. MCA-205 cells one (**A**) and two (**B**) days after thawing in a T75 flask. Cells express vimentin (**C,** Cat. No. AB5733), a marker of fibrosarcoma, and actin (**D,** Sigma Cat. No. P5282). Cell nuclei are stained with Dapi (blue).

References

1. *Oncotarget.* 2017; 8(61): 104638-104653.
2. *Cancer Res..*1996; 56(19): 4338-4342.
3. *J Immunol.* 1990; 144(4): 1531-1537.
4. *J Immunol.* 1988; 140: 2453-2461.

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Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium and flasks are on hand.

   MCA-205 Expansion Medium: Cells are thawed and expanded in RPMI-1640 medium (Sigma Cat. No. R0883) containing 2 mM L-glutamine (Cat. No. TMS-002-C), 1 mM sodium pyruvate (Cat. No. TMS-005-C),10% FBS (Cat. No. ES-009-B), 1X non-essential amino acids (Cat. No. TMS-001-C), and 1X β-mercaptoethanol (Cat. No. ES-007-E).
2. Remove the vial of frozen MCA-205 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 MCA-205 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 15 mL of MCA-205 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.

Subculturing Cells

1. Passage cells at 80% confluence at a 1:6 dilution.
2. Carefully remove the medium from the T75 tissue culture flask containing MCA-205 cells.
3. Rinse the flask once with 10 mL 1X PBS. Aspirate after the rinse.

   Note: Cells are loosely attached and may detach easily. Be careful during the rinse.
3. Apply 5-7 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 5-7 mL of MCA-205 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 2-5 mL of MCA-205 Expansion Medium 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. Initially conservative splitting (such as 1 to 2 or 1 to 3) may help cells grow better. Once established, typical split ratio is 1:6.

Cryopreservation of Cells

MCA-205 murine fibrosarcoma cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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