AT-3 Mouse Mammary Carcinoma Cell Line

Tumor Cell Line

Cat. # SCC178

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:

Tumor-induced immunosuppression is a common phenomenon in cancer and an important consideration for cancer immunotherapy. However, most studies on tumor-induced immunosuppression rely on tumor implant models, in which the disease progresses rapidly and usually causes non-physiological host responses that do not recapitulate normal tumor development and progression.[1] Models that generate autochthonous tumors are therefore valuable for providing more physiologically-relevant systems for investigating tumor-host interactions and longer-term immunosuppressive effects.

The AT-3 cell line was isolated from an autologous mammary tumor from cells originating in a transgenic MTAG mouse model.[2] Mammary tumors arising in MTAG mice closely mimic changes observed in human breast carcinomas over the course of disease progression.[3] AT-3 cells are a useful mouse model for studies of tumor physiology that are representative of typical disease states in humans.

Source

The AT-3 cell line was derived from primary mammary tumor cells of a transgenic MTAG mouse. The MTAG mouse model expresses the polyoma virus middle T antigen under control of the MMTV-LTR promoter.[4]

Note: AT-3 cells demonstrate diverse morphologies in culture.

Storage and Handling

AT-3 Mouse Mammary Carcinoma 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

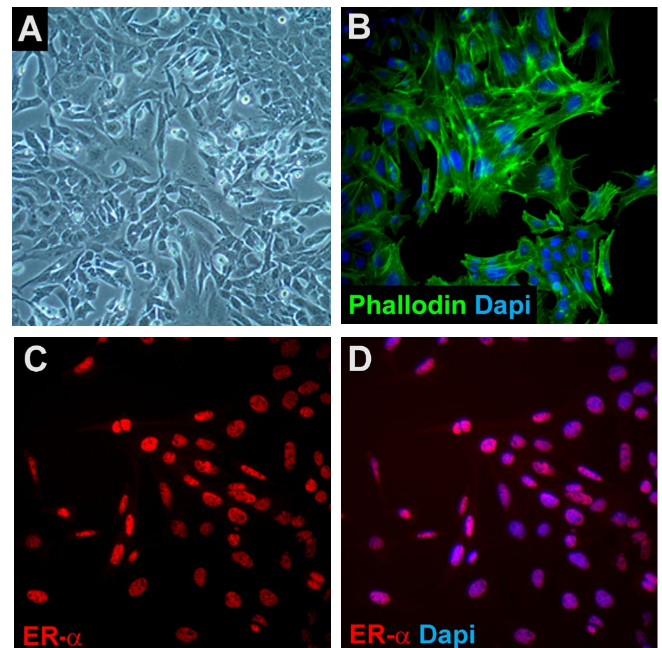

Figure 1. AT-3 cells two days after thawing in a T75 flask (**A**, 10X magnification). Cells express actin (Phalloidin, **B**, green) and estrogen receptor alpha (ER-α, **C**, **D**, red). Expression of ER-α is localized to cell nuclei. Cell nuclei are stained with Dapi (blue).

References

1. *Methods Mol Med.* 2005; 111: 335-350.
2. *J Immunol.* 2007; 179(5): 2851-2859.
3. *Am J Pathol.* 2003; 163(5): 2113-2126.
4. *Mol Cell Biol.* 1992; 12(3): 954-961.

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Protocols

Thawing Cells

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

   AT-3 Expansion Medium: Cells are thawed and expanded in DMEM-High Glucose medium containing L-glutamine and sodium pyruvate (Sigma Cat. No. D6429) supplemented with 10% heat-inactivated FBS (Cat. No. ES-009-B), 2 mM non-essential amino acids (Cat. No. TMS-001-C), 15 mM HEPES, and 1X β-mercaptoethanol (Cat. No. ES-007-E).
2. Remove the vial of frozen AT-3 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 AT-3 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 AT-3 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 AT-3 cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after 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 AT-3 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 AT-3 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. Typical split ratio is 1:6.

Injection into Mice

Cells are injected subcutaneously in the mammary fat pad. Approximately 200,000 cells in 100 μL per mouse.

Cryopreservation of Cells

AT-3 mouse breast tumor cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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