MEC1 Mouse Embryonic Epicardial Cell Line

Immortalized Cell Line

Cat. # SCC187

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:

Heart development is guided by mitogenic factors secreted by the epicardium, a cell layer that overlays the myocardium in the mid-gestation embryonic heart. Despite the importance of these factors in directing cardiomyocyte proliferation and morphogenesis, they are not well-characterized. Cellular models of the embryonic epicardium are thus essential for gaining a comprehensive understanding of the mechanisms of cardiac development.

MEC1 is an immortalized mouse embryonic epicardial cell line that does not express myocardial or endothelial cell lineages. MEC1 cells retain the morphology of early primary epicardial cells and express epicardium-specific markers including epicardin (Tcf21), Tbx18 and cytokeratin-18. MEC1 cells localized ZO-1 and beta-catenin in adherens junctions at the cell membrane and possessed organized actin cytoskeletal filaments. The native physiological features of the MEC1 cell line contribute to its suitability for addressing a wide range of questions in cardiac developmental biology.

Source

Non-GMO. MEC1 is a stable cell line derived from primary cultures of E13.5 wild-type embryonic ventricles of an ICR/CD1 strain mouse. Individual colonies that retained early epicardial cell morphology underwent several iterative rounds of subcloning.

Storage and Handling

MEC1 Mouse Embryonic Epicardial 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

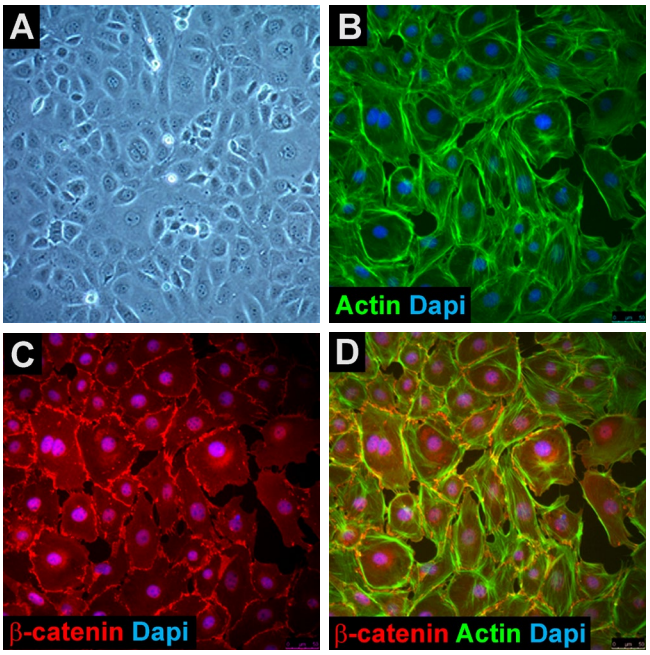

Figure 1. MEC1 cells one day after thawing in a T75 flask (**A**, 10X magnification). Cells express actin (Phalloidin, **B**, **D**, green) and β-catenin (**C**, **D**, red). B-catenin is highly visible at cell junctions. Cell nuclei are stained with Dapi (blue).

References

Li P, Cavallero S, Gu Y, Chen TH, Hughes J, Hassan AB, Brüning JC, Pashmforoush M, Sucov HM (2011). IGF signaling directs ventricular cardiomyocyte proliferation during embryonic heart development. *Development* 138(9): 1795-17805.

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Protocols

ECM Coating of Flasks

1. Coat culture flasks with 0.1% gelatin solution (Cat. No. ES-006-B) for a minimum of 30 minutes at room temperature. Use 10 mL for T75 flasks. For T175 flasks, use 15 mL volume. For T225 flasks, use 20-25 mL volume.

   Note: Flasks may be coated 5-6 days in advance and stored at 2-8°C in the coating solution.
2. Aspirate the coating solution just before plating the cells.

Thawing Cells

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

   MEC1 Expansion Medium: Cells are thawed and expanded in DMEM-High Glucose (Cat. No. SLM-120-B) supplemented with 10% FBS (Cat. No. ES-009-B).
2. Remove the vial of frozen MEC1 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 MEC1 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 MEC1 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 75% confluence (usually every 2-3 days) at a 1:6 dilution. Use gelatin coated plates.
2. Carefully remove the medium from the T75 tissue culture flask containing MEC1 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 MEC1 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 MEC1 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.

Cryopreservation of Cells

MEC1 mouse embryonic epicardial cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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