EpH4 Mouse Mammary Epithelial Cell Line

Immortalized Cell Line

Cat. # SCC284

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:

Mammary epithelial cells are a model for cellular plasticity, the ability to undergo varied morphological changes. One of the significant aspects of mammary-derived tumors is epithelial-to-mesenchymal transition (EMT), which gives rise to the migratory behavior required for metastasis.[1] Cellular plasticity makes mammary epithelial cell models valuable for the study of breast cancer malignancy in addition to hormonal regulation of differentiation and epithelial cell polarization.

Eph4 is a spontaneously immortalized cell line derived from primary murine mammary epithelial cells.[2] EpH4 cells can be induced to synthesize milk proteins, such as caseins, upon the induction with lactogenic hormones such as prolactin, hydrocortisone, and insulin. For normal maintenance Eph4 cells should be cultured at high density (semi-confluency) and passaged before reaching confluency to avoid polarization and selective culture of single cells. Post-confluent Eph4 cells undergo polarization and formation of hemicysts or dome-like structures characterized by unidirectional transport of ions.[3] The Eph4 cell line is a versatile system for studying a spectrum of morphological transformation spanning breast cancer metastasis, EMT, and hormonal regulation.

Source

Eph4 cell line is a spontaneously immortalized clone derived from primary mammary epithelial cells of a female BALB/c mouse.

Storage and Handling

EpH4 Mouse Mammary Epithelial 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

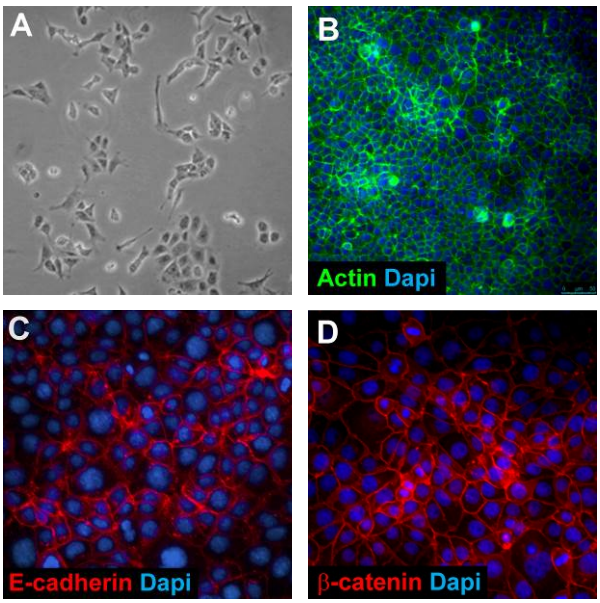

Figure 1. Bright-field image of cells one (A) day after thaw. Cells express Actin (**B**, Sigma P5282), E-cadherin (C, Abcam AB231303) and β-catenin (D, Sigma ABE208).

References

1. *Breast Cancer Res.* 2005; 7(4): 171-179.
2. *EMBO J* 1995; 14(6): 1145-55.
3. *J Cell Biol.* 1989; 108(3): 1127-38.

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Protocol:

For expansion, it is important to permanently keep EpH4 cells in a non-polarized state. To maintain the properties of EpH4 cells, it is important to seed them at relatively high cell density, which is about 35,000 cells/cm2 (semi-confluent). Any conditions that would lead to a cloning of single cells have to be avoided. After 3 days in culture, the cells should be confluent again. It is recommended to passage the cells at a plating density of 35,000 – 40,000 cells/cm^2.

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 expanded in DMEM-High Glucose (Sigma Cat. No. D5796) and 10% FBS (Cat. No. ES-009-B).
2. Remove the vial of frozen EpH4 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 EpH4 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 EpH4 Expansion Medium and 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. Allow the cells to grow to confluence. Aspirate and rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 mL of Accutase 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 EpH4 Expansion Medium to the plate. 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. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of EpH4 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. Plate the cells at a 1:3 or 1:4 split ratio onto a similarly sized tissue culture vessel. Recommended plating density is 35,000 – 40,000 cells/cm^2.

Dome-formation on cell culture plastic: Dependent on their polarization state, EpH4 cells may start to form domes about 5- days after they have reached confluency. Initially small domes are visible and then increase over time. It is not recommended to passage the dome cultures for routine expansion as cell sheets and clumps (rather than single cells) will then settle down on a new culture plate, resulting in the formation of a non-homogeneous monolayer. Single cells and cell clusters may greatly differ in their polarization state, resulting in a non-homogenous monolayer.

Formation of ducts and end-buds in collagen I hydrogels:

Submerge EpH4 cells in 1-2 mL of freshly neutralized Collagen I (initial concentration ~5 mg/mL) and transfer onti a 3 cm diameter poly-carbonate filter insert or on plastic culture dishes. Avoid air bubbles when pipetting the collagen. Make sure the cells remain “trapped” in 3 dimensions in the polymerizing hydrogel.

Cryopreservation of Cells

EpH4 Mouse Mammary Epithelial Cell Line may be frozen in EpH4 Expansion Medium and 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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