Data Sheet

PMC42-LA Human Breast Cancer Cell Line

Cancer Cell Line

SCC139

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.

Background

Human breast carcinoma cell lines are useful models to investigate the pathogenesis of neoplastic transformation and aid in the development of novel therapeutic strategies. Nonetheless, there are few well characterized cell lines derived from human mammary carcinomas and loss of breast-specific marker expression and the emergence of dedifferentiated phenotypes over extended culture often limit their use.[1]

Initiated from a pleural effusion of a metastatic breast cancer patient, the human mesenchymal breast carcinoma cell line PMC42 (also known as PMC42ET), is unique in that these cells remained pleomorphic even after several years of sustained culture.[2,3] Typically grown in monolayer, PMC42 continue to give rise to numerous morphologically different cell types of both secretory and myoepithelial characteristics and this retained ability to differentiate *in vitro* earned their designation as stem cell like.[2,3]

PMC42-LA is a stable epithelial variant of the mesenchyme-like parental PMC42/PMC42-ET cell line. PMC42-LA congregate into pavement epithelial sheets at high density, in which the cell-cell borders contain E-cadherin and β-catenin, albeit 5-10% of cells do express the mesenchymal marker vimentin. When stimulated with epidermal growth factor (EGF), PMC42-LA cells undergo an epithelial-mesenchymal transition in which vimentin expression is upregulated and E-cadherin expression is reduced.[5]

Short Tandem Repeat

D3S1358: 15	D16S539: 9, 11
TH01: 6	CSF1PO: 10, 12
D21S11: 30, 31.2	Penta D: 12, 13
D18S51: 14, 17	vWA: 15, 19
Penta E: 11, 12	D8S1179: 13
D5S818: 11, 12	TPOX: 8, 11
D13S317: 11, 14	FGA: 20
D7S820: 7, 9	Amelogenin: X

Cancer cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- Each vial contains ≥ 1x10^6 viable cells.

- Cells are tested by PCR and are negative for HPV-16, HPV-18, Hepatitis A, C, and HIV-1 & 2 viruses.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Storage and Handling

PMC42-LA Human Breast Cancer 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.

Representative Data

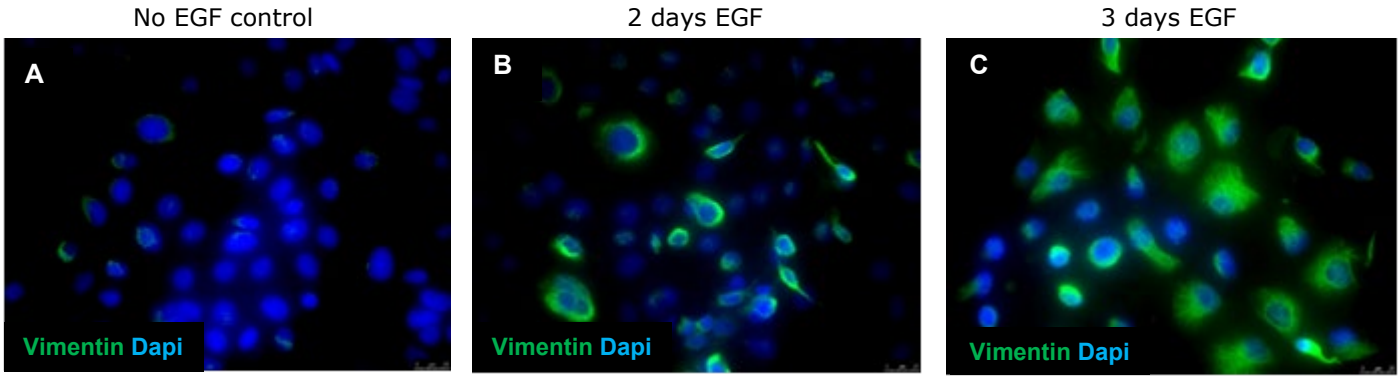

Figure 1. (A) Without EGF treatment, only 5% to 10% of PMC42-LA cells express vimentin. In response to EGF, PMC42-LA cells underwent EMT-like changes including acquisition of vimentin expression; (**B**) 2 days; (**C**) 3 days). Nuclei were stained with DAPI (blue).

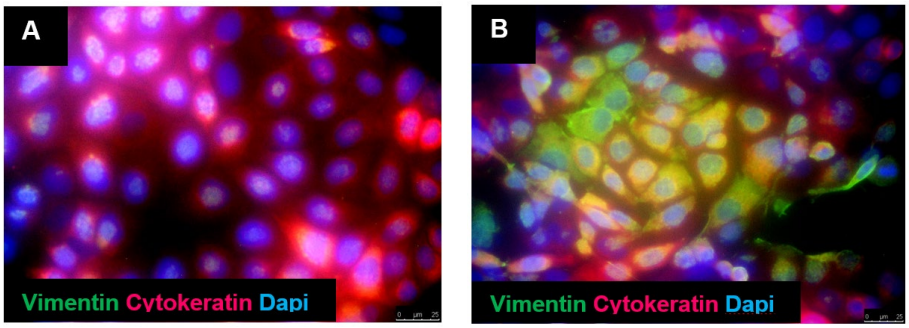

Figure 2. Double staining with pan-cytokeratin antibody (red) and vimentin (green) indicate that (**A**) without EGF treatment, most cells were keratin positive and vimentin negative. (**B**) After EGF treatment for 3 days, most cells were positive for both keratin and vimentin. Nuclei were stained with DAPI (blue).

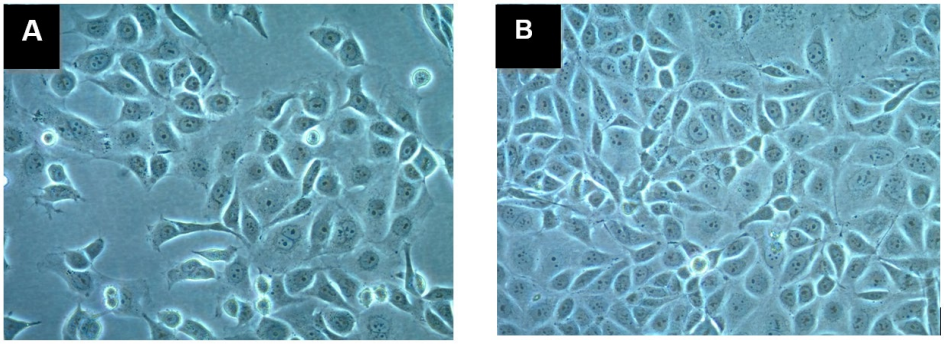

Figure 3. Typical morphology of the PMC42-LA Human Breast Cancer Cell Line (**A**) 24 hours and (**B**) 48 hours after thaw.

Protocols

Thawing of Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue culture ware surfaces without any additional coating.

   Cells are thawed and expanded in RPMI-1640 (R8758-500ML) containing 10% FBS (ES-009-B).
2. Remove the vial of frozen PMC42-LA 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 1-2 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 PMC42-LA Expansion Media (Step 1 above; pre-warmed to 37 °C) 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 10-15 mL of PMC42-LA 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.
12. The next day, exchange the medium with 10-15 mL of fresh PMC42-LA Expansion Medium pre-warmed to 37 °C. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 90% confluent, they can be dissociated with Accutase™ (SCR005) or trypsin-EDTA (SM-2003-C) and further passaged or, alternatively, frozen for later use.

Subculturing of Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of C8/I2 cells.
2. Apply 3-5 mL of Accutase™ or trypsin-EDTA solution and incubate in a 37 °C incubator for 3-5 minutes.
3. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.

4. Add 8 mL of 10% FBS Medium (pre-warmed to 37 °C) to the plate.
5. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
7. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
8. Apply 2 mL of 10% FBS Medium (pre-warmed to 37 °C) to the conical tube and resuspend the cells thoroughly.
   Important: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density (typical split ratio is 1:8 to 1:10).

Cryopreservation of Cells

PMC42-LA cells can be frozen in the expansion media plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Engel LW, Young NA (1978) Human breast carcinoma cells in continuous culture: a review. Cancer Res. 38(11 Pt 2):4327-4339.
2. Whitehead RH, Bertoncello I, Webber LM, Pedersen JS (1983) A new human breast carcinoma cell line (PMC42) with stem cell Characteristics. I. Morphologic characterization. J Natl Cancer Inst 70 (4): 649-661.
3. Whitehead RH, Monaghan P, Webber LM, Bertoncello I, Vitali AA (1983) A new human breast carcinoma cell line (PMC42) with stem cell characteristics II. Characterization of cells growing as organoids. J Natl Cancer Inst 71(6): 1193-1203.
4. Ackland ML, Michalczyk A, Whitehead RH (2001) PMC42, a novel model for the differentiated human breast. Exp Cell Res 263(1): 14–22.
5. Ackland ML, Newgreen DF, Fridman M, Waltham MC, Arvanitis A, Minichiello J, Price JT, Thompson EW (2003) Epidermal growth factor-induced epithelio-mesenchymal transition in human breast carcinoma cells. Lab Invest 83(3): 435–448.

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