Data Sheet

Py2T Mouse Mammary Tumor Cell Line

SCC416

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

Py2T is a stable murine cancer cell line derived from the mammary tumor of MMTV-PyMT (mouse mammary tumor virus-polyoma middle tumor-antigen) transgenic mouse.[1] PyMT00153749 Ver 1.0, Rev 17NOV2023, RC, AB is an oncogene which transforms cells in culture and leads to formation of mammary tumor in MMTV-PyMT mice by interacting with non-receptor tyrosine kinase, c-Src.[2] These cells have a uniform cobblestone-like morphology, typical of differentiated epithelial cells, and express estrogen receptor (ER), both basal (CK14) and luminal (CK8/18) markers. When these cells are implanted orthotopically in syngeneic FVB/N mice they form non-metastatic invasive tumors with low or absent E-cadherin.[1]

Ninety percent of breast cancer-related deaths are ascribed to systemic dissemination of cancer cells leading to their metastatic outgrowth in distant organs. Epithelial-mesenchymal transition (EMT) is a process involved in the initiation of the invasion-metastasis cascade of cancer cells.[3] Py2T undergo epithelial-mesenchymal transition (EMT) in response to TGFβ *in vitro*. Stimulation with TGFβ was reported to induce the canonical pathway as evidenced by nuclear translocation of the Smad2/3 complex and phosphorylation of Smad3.[1] However, these cells are resistant to TGFβ-induced cell cycle arrest and utilize non-canonical TGFβ signaling to induce EMT.[3] Long term treatment with TGFβ (2 ng/mL) leads to formation of a mesenchymal subline of cells (Py2T LT) with elongated morphology expressing mesenchymal markers such as vimentin, fibronectin and N-cadherin. Orthotopic implantation of Py2T LT cells has been reported to form microscopic lung metastatic lesions in 50% of mice.[4] These cell lines will serve as a versatile model to investigate the molecular mechanisms underlying EMT, a prerequisite in the early steps of metastasis, as well as mesenchymal epithelial transition.

Source

Py2T cell line was derived from a mammary tumor of a MMTV-PyMT transgenic mouse.[1]

Short Tandem Repeat

M18-3: 17	M1-2: 13,17	M8-1: 15	M11-2: 15	MX-1: 26
M4-2: 19.3	M7-1: 29	M2-1: 9	M17-2: 15	M13-1: 15.2
M6-7: 12	M1-1: 10,11	M15-3: 20.3,	M12-1: 20	
M19-2: 12	M3-2: 14	M6-4: 15.3	M5-5: 14	

Quality Control Testing

- Py2T Mouse Mammary tumor cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

Py2T mouse mammary tumor cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

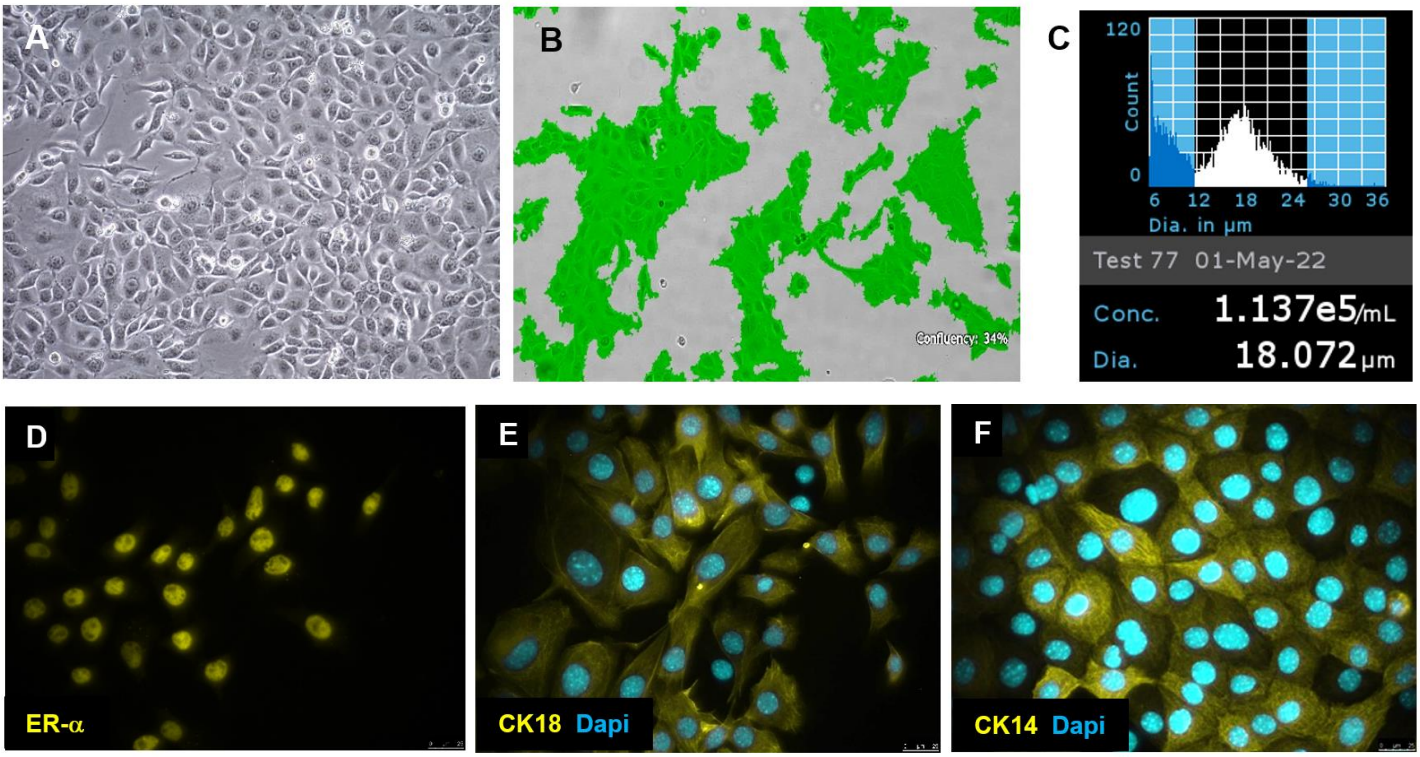

Figure 1. **A**. Brightfield image of Py2T cells one day after thaw into a T75 flask. **B**. Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). **C**. Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter (PHCC360KIT) using 60 µm sensors. **D**. Py2T cells express estrogen receptor alpha, ER-α (06-935), and luminal and basal markers–**E**. CK18 (MAB3234) and **F**. CK14 (ZRB1441), respectively.

NOTE: Product catalog numbers indicated in ( ) can be purchased at SigmaAldrich.com unless otherwise stated.

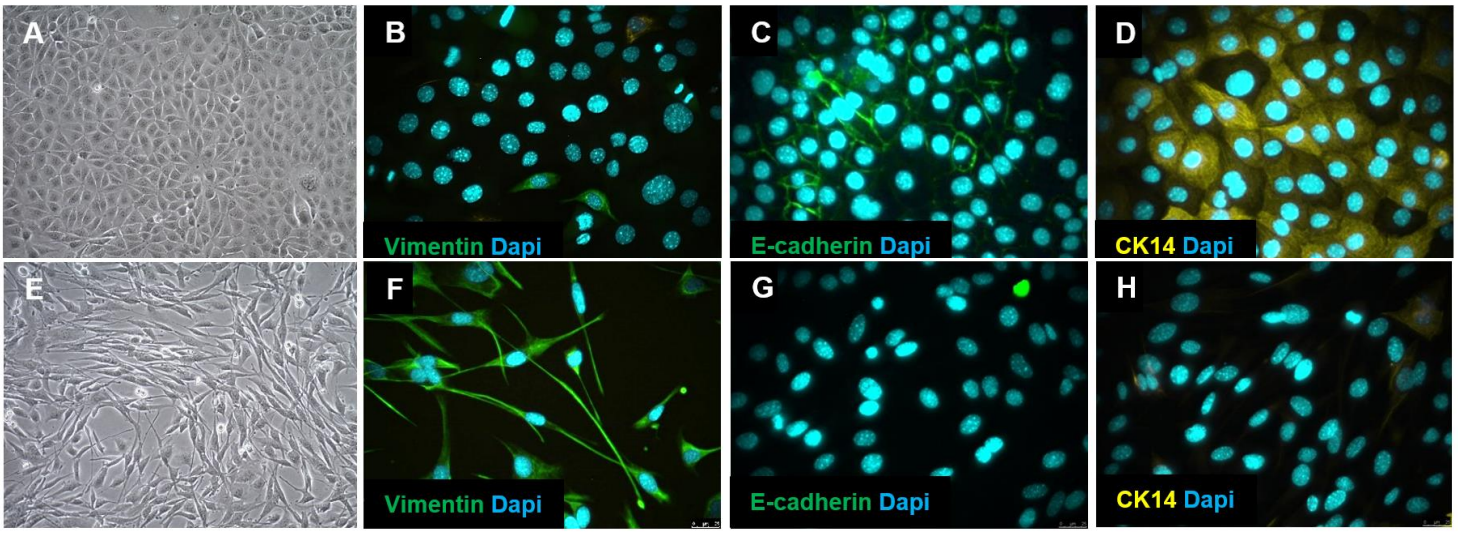

Figure 2. **E**–**H**. Py2T cells were treated with 2 ng/mL TFGb (GF346) for 20 days to form the mesenchymal subline. **A**–**D**. Untreated control Py2T cells. **B**. Immunofluorescence image (40X) depicting the expression of vimentin. **F**. (AB5733) in the mesenchymal subline relative to untreated control. **C**. (LifeTechnologies 13-1900) Untreated control Py2T cells express E-cadherin and **D**. (ZRB1441) CK14 whereas the 2 ng/mL TGFb treated mesenchymal subline does not stain for **G**. E-cadherin or **H**. CK14.

Protocols

Thawing the Cells

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

   Py2T cells are thawed in Py2T Expansion Medium comprised of DMEM-High Glucose medium (D5671) containing 10% FBS (ES-009-B), 2 mM L-Glutamine (G7513) and Pen/Strep (P4333).
2. Remove the vial of frozen Py2T 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 4 mL of PY2T 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 5 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 Py2T Expansion Medium.
10. Transfer the cell mixture to a T175 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. Do not allow the cells to grow to confluency. Py2T cells can be passaged at ~85% to 90% confluency.
2. Carefully remove the medium from the T175 tissue culture flask containing the 85% confluent layer of Py2T cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed 0.25% Trypsin (SM-2003-C) and incubate in a 37 °C incubator for 5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of Py2T Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 8 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of Py2T expansion medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

Py2T cells may be frozen in Py2T cell expansion Medium (without Pen/Strep) supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. PLoS One 2012; 7(11): e48651. PMID: 23144919.
2. Oncogene 2015; 34(32):4190-4198. PMID: 25362852.
3. Sci Rep 2018; 8(1):12123. PMID: 30108334.
4. Ayse NK. University of Basel 2017; Dissertation. https://edoc.unibas.ch/59030/1/Ph.D.%20Dissertation.pdf.

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