Data Sheet

Lacun.3 Mouse Lung Adenocarcinoma Cell Line

Cancer Cell Line

SCC624

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

Recent cancer research often studies a newly characterized type of cell referred to as cancer stem cells (CSCs) or also sometimes termed tumor-initiating cells (TICs.) These cells appear to have significant capabilities to grow, differentiate, generate tumors and resist chemotherapy.[1] Tumor sphere cultures (TSCs) have emerged as a new format of studying cancer as well as understanding CSCs. The spheroid structures of TSCs appear to induce stem-like characteristics evidenced by the increased expression of stem cell markers. This 3D spheroid structure is also a much more accurate way to mimic the behavior of a solid tumor which develops in 3-dimensions and behaves quite differently from a monolayer cell culture. Spheroids are understood to have more tumor-forming capabilities when compared to monolayer cultures, yet the underlying mechanism for these characteristics is still under study. Stem-cell like properties, such as increased aldehyde dehydrogenase activity (ALDH), have already been used to identify CSCs in a variety of cancer types.[3]

The Lacun.3 cell line was obtained from a chemically induced lung adenocarcinoma which developed in mice.[1] This cell line is capable of forming spheroids when cultured in the appropriate media. The spheroid culture can be maintained over multiple passages and displays increased stem-cell characteristics including increased ALDH activity when compared to adherent cultures using the same line. TSC cultured Lacun.3 shows high tumor formation when injected into mice although metastatic capabilities appear to be weakened. While TSC Lacun.3 growth characteristics are slower compared to adherent culture, their chemoresistance capabilities were higher. In addition, a drug that was screened to specifically kill CSCs had much higher success in killing TSC Lacun.3 cells, indicating their CSC phenotype. Furthermore, the Lacun.3 cell line provides a useful model for studying CSCs by spheroid formation.

Source

Non-GMO. Cell line is derived from a chemically induced lung adenocarcinoma developed in a mouse model.

Short Tandem Repeat

M18-3: 18	M4-2: 21.3	M6-7: 12	M19-2: 14	M1-2: 17	M7-1: 24.2, 26
M1-1: 14	M3-2: 14	M8-1: 13	M2-1: 16	M6-4: 17	M15-3: 16, 22.3
M11-2: 17	M17-2: 18	M12-1: 16	M5-5: 14	MX-1: 25	M13-1: 16.2, 17.2

Representative Data

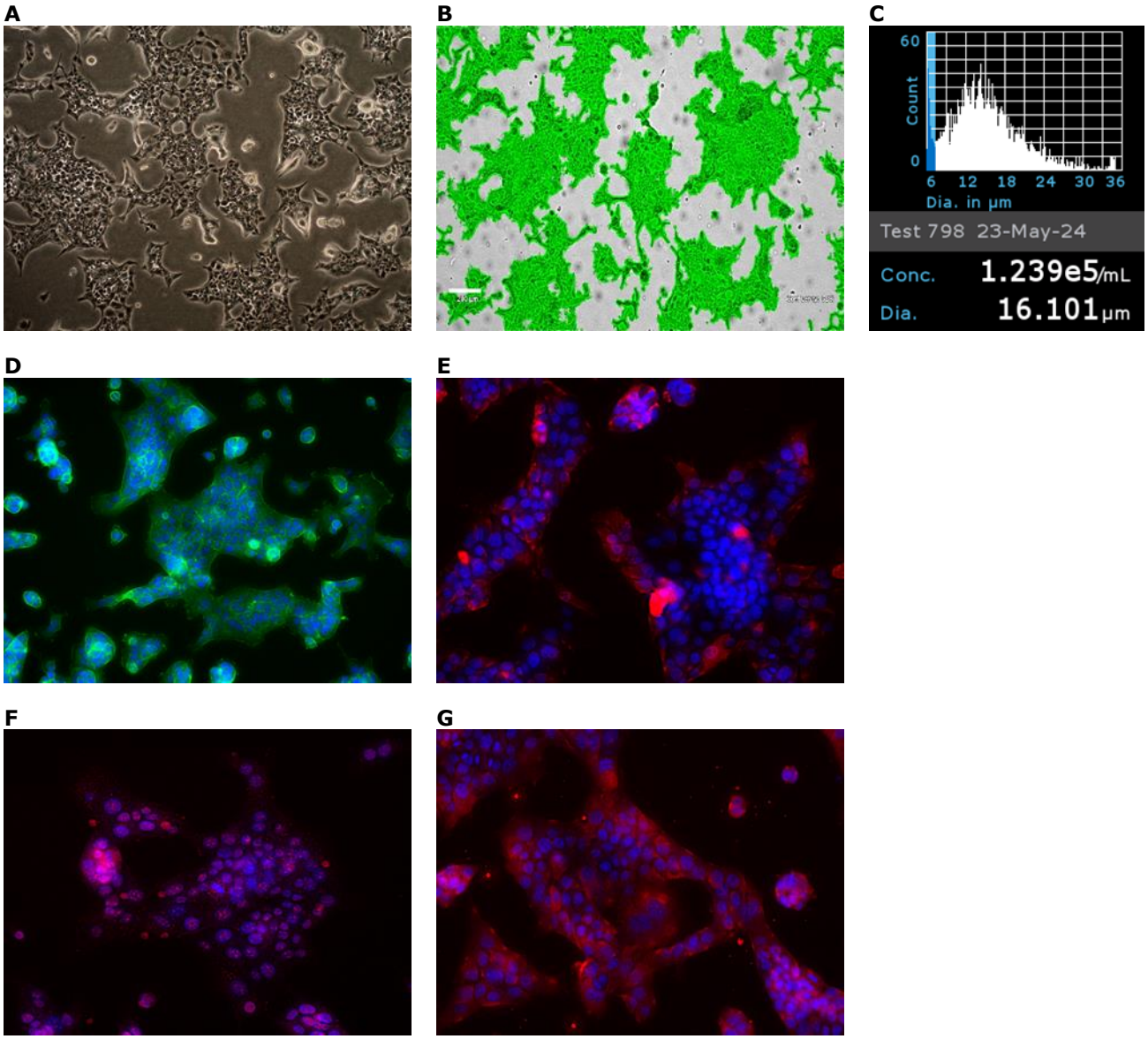

Figure 1. (**A**) Bright-field images of Lacun.3 cells three days after thaw in a T175 flask (4X magnification). (**B**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C**) Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors (PHCC360KIT). (**D**) Lacun.3 cells stained with Phalloidin-Atto-488 (49409). (**E**) Lacun.3 cells express ALK protein (ZRB1937). (**F**) Lacun.3 cells express TTF1 protein (HPA054837). (**G**) Lacun.3 cells express SCA-1 (ZRB2309).

Quality Control Testing

- The Lacun.3 Mouse Lung Adenocarcinoma cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and nonhuman 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

Lacun.3 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.

Protocols

Thawing the Cells

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

1. Lacun.3 cells are thawed and expanded in Lacun.3 Expansion Medium comprising of RPMI1640 (R8758) containing 10% FBS (ES-009-B), 2 mM L-Glutamine (G7513) and Penicillin/Streptomycin (P4333) (optional).

   Note: Spheroid culturing requires a separate media listed in "Spheroid Culturing" section below.
2. Remove the vial of frozen Lacun.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 Lacun.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 35 mL of Lacun.3 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. The Lacun.3 cells can be passaged at ~80-85% confluency.
2. Carefully remove the medium from the tissue culture flask containing the 80-85% confluent layer of Lacun.3 cells.
3. Rinse the flask with 10 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® (A6964) 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 Lacun.3 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 3-5 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 Lacun.3 cell 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 1:10.

Spheroid Culturing

1. Cells can be cultured in a spheroid format by dissociating cells into single suspension followed by seeding onto an ultra-low attachment plate (M96ULA, CLS3814, CLS3261).
2. Spheroid formation requires culturing in tumor spheroid culture (TSC) media which consists of DMEM/F12 (DF-042-B) supplemented with B27 (ThermoFisher 17504044), hEGF, Insulin, hydrocortisone, and GA-100 (Fisher, NC9742592).
3. Spheroids form readily after seeding. Spheroids can be cultured for multiple passages. Media changes should be conducted every 3-5 days and cells should be passaged every 5-7 days.

Cryopreservation of the Cells

The Lacun.3 cells may be frozen in Lacun.3 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Bleau A-M, Zandueta C, Redrado M, Martínez-Canarias S, Larzábal L, Montuenga LM, Calvo A, Lecanda F. 2015. Sphere-derived tumor cells exhibit impaired metastasis by a host-mediated quiescent phenotype. Oncotarget. 6(29):27288–27303. doi:https://doi.org/10.18632/oncotarget.4803. [accessed 2024 Apr 11]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4694990/pdf/oncotarget-06-27288.pdf.
2. Weiswald L-B, Bellet D, Dangles-Marie V. 2015. Spherical Cancer Models in Tumor Biology. Neoplasia. 17(1):1–15. doi:https://doi.org/10.1016/j.neo.2014.12.004.
3. Toledo-Guzmán ME, Hernández MI, Gómez-Gallegos ÁA, Ortiz-Sánchez E. 2019. ALDH as a Stem Cell Marker in Solid Tumors. Current Stem Cell Research & Therapy. 14(5):375–388. doi:https://doi.org/10.2174/1574888x13666180810120012.

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