Data Sheet

Calu-3 Human Lung Adenocarcinoma Cell Line

Cancer Cell Line

SCC438

Pack Size ≥1x10^6 viable cells/vial

Store at: Liquid Nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Lung cancer is the most common culprit of cancer-related deaths in the world.[1] Adenocarcinoma, which begins in the mucus-producing cells of the body, is the most common sub-categorization of primary lung cancer. It accounts for about 40% of all cases of lung cancer.[2] Adenocarcinoma is aggressive and is usually diagnosed at more advanced stages, resulting in low survival rates.

Adenocarcinoma presents a variety of challenges due to its resistance to common treatments such as radiotherapy and chemotherapy. Combining multiple forms of therapy helps to overcome these roadblocks. The Calu-3 adenocarcinoma model is a valuable tool for identifying potential drug therapies and has proven to be useful for mimicking *in vivo* conditions.[3] This utility has facilitated pharmaceutical applications such as drug transport, studying aerosol deposition, controlled release studies, and identification of potential drug-drug interactions.[4] The characteristics of the Calu3 cell line allow for a fundamental and essential *in vitro* method for developing new therapeutics in an epithelial respiratory model.

Source

The Calu-3 cell line was established by isolating cells located in the pleural effusion of a 25-year-old Caucasian man with lung adenocarcinoma.[5]

Short Tandem Repeat

D3S1358: 15, 18	D18S51: 14, 17	TPOX: 8
D7S820: 10, 11	D5S818: 11	CSF1PO: 11, 12
vWA: 16, 17	D13S317: 12	Amel: X
FGA: 25	D16S539: 12, 14	Penta D: 9, 16
D8S1179: 11, 15	TH01: 6, 9.3	Penta E: 5, 21
D21S11: 28, 30		

STR Cancer cell lines are inherently genetically unstable. 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 negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from mouse, rat, Chinese hamster, Golden Syrian hamster, and Non-human Primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

Calu-3 cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Representative Data

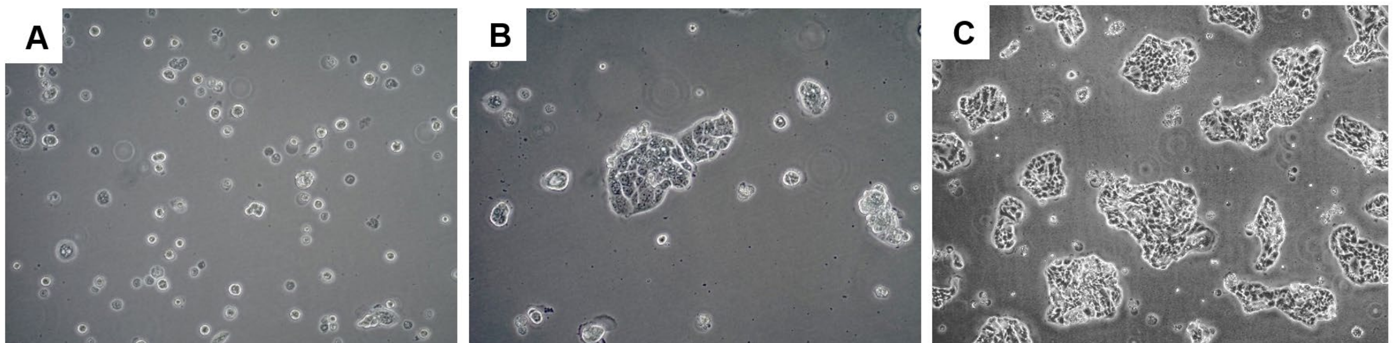

Figure 1. Bright-field images of Calu-3 cells in culture, one (**A**) and four (**B**) days after thaw in a T75 flask. Calu-3 cells grow as cell clusters (**C**).

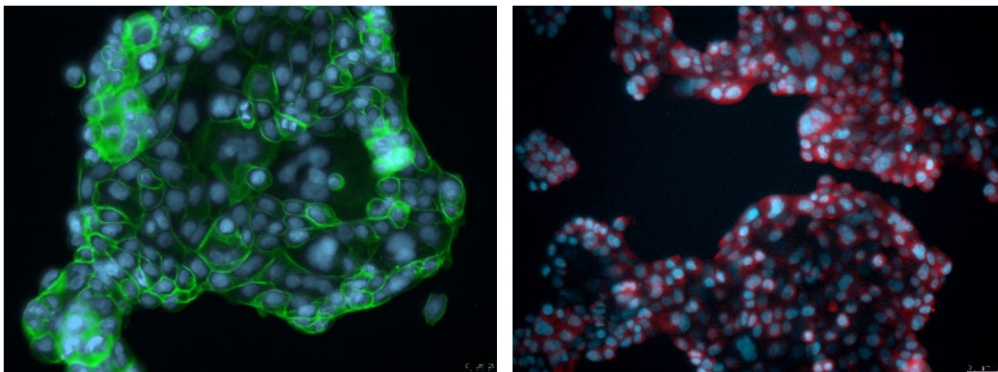

Figure 2. Calu-3 express actin, green (**A**, Cat. No. P5282) and connexin 43, red (**B**, Cat. No. MABT528-AF488). Blue, DAPI.

Protocols

Thawing Cells

Cell viability of Calu-3 cells upon thawing may be less than 80% but should be at least 70% viable. Calu-3 cells proliferate relatively slowly and are prone to cell clusters and island formation when the culture reaches 35-40% confluence.

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 Calu-3 Expansion Medium comprised of Minimal Essential Medium Eagle (Cat. No. M2279) with 2 mM L-Glutamine (Cat. No. TMS-002-C), 10 mM HEPES (Cat. No. TMS-003-C) and 10% FBS (Cat. No. ES-009-B).
2. Remove the vial of frozen Calu-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 Calu-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 15 mL of Calu-3 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. Calu-3 cells should be passaged at ~80-85% confluency. Do not allow the cells to grow over 85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of Calu-3.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase™ and incubate in a 37 °C incubator for 3-5 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 10 mL of Calu-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 Calu-3 Expansion Medium to the conical tube and resuspend the cells thoroughly.

    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

Calu-3 cells may be frozen in Calu-3 Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Semin Ultrasound CT MR 2019, 40(3): 255-264.
2. Cell Death Dis 2018, 9(2): 117.
3. J Control Release 2003, 87(1-3): 131-138.
4. Expert Opin Drug Deliv 2013, 10(9): 1287-1302.
5. J Natl Cancer Inst 1977, 59(1): 221-226.

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