Data Sheet

T24T Human Bladder Carcinoma Cell Line

Cancer Cell Line

SCC468

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

Bladder cancer originates from the epithelium that covers the inner surface of the bladder. The most common type is urothelial or transitional carcinoma.[1] Most of the newly diagnosed cases (75%) are non-invasive. When tumors becomes invasive, they are more likely to metastasize to lymph nodes and other organs. Two common alterations have been identified in non-invasive tumors: deletion of chromosome 9 and a point mutation in FGFR3 (Fibroblast growth factor receptor 3).[2] Activating mutations in RAS gene family members are also common in bladder carcinoma, and it is estimated that activation of the RAS-MAPK pathway may contribute to over 80% of the non-invasive cases.[1] Loss of function of tumor suppressor genes like TP53 and RB1 are common in cases of invasive disease.[1] After diagnosis, resection of the tumor is common. In cases of invasive disease, chemotherapy is typically recommended.

The original cell line T24 has been used to study bladder tumor biology but has some limitations because it lacks tumorigenicity when injected into immunocompromised mice. In contrast, T24T has increased metastatic ability when tested *in vivo* and expresses higher levels of HRAS mRNA.[3] Increased HRAS activity leads to focal adhesion disassembly and loss of CDH1 and β-catenin from intercellular junctions.[3] Contact inhibition growth is reduced in T24T compared with T24.[3] Additionally, T24T motility is not inhibited at higher density, as has been reported with the T24 line.

Source

T24 cell line was generated from an invasive human transitional cell carcinoma of the bladder.[4] T24T is a derivative of T24.

Short Tandem Repeat

D3S1358: 16	D18S51: 16, 18	TPOX: 8, 11
D7S820: 10, 11	D5S818: 10, 12	CSF1PO: 10, 12
vWA: 17	D13S317: 12	Amel: X
FGA: 22	D16S539: 9	Penta D: 11, 15
D8S1179: 14	TH01: 6	Penta E: 7, 10
D21S11: 29		

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

T24T 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

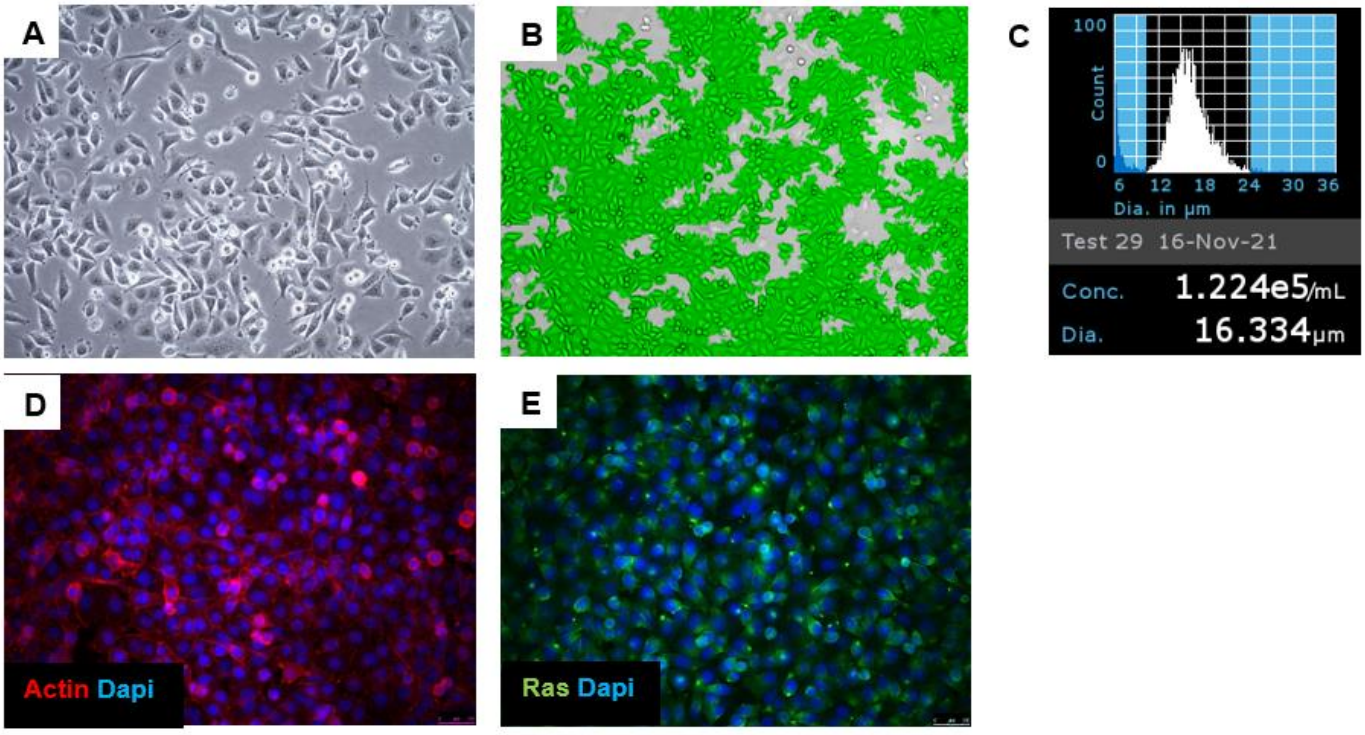

Figure 1. Bright-field image of T24T cells one day after thaw in a T75 flask (**A**). Cell confluency (81%) was assessed throughout the culture using MilliCell® Digital Cell Imager (**B**, Cat. No. MDCI 10000). Cell counting was performed using Scepter™ 3.0 handheld automated cell counter using 60 µm sensors (**C**, PHCC360KIT). T24T cells express actin (**D**, red, Cat. No. 49409) and Ras (**E**, green, Cat. No. MABS195).

Protocols

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.
2. Cells are thawed and expanded in T24T Expansion Medium comprised of DMEM/F12 medium (Cat. No. D8062) and 5% FBS (Cat. No. ES-009-B).
3. Remove the vial of frozen T24T 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.
4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
5. 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.
6. Using a 10 mL pipette, slowly add dropwise 9 mL of T24T 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.
7. 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.
8. Centrifuge the tube at 300 *x g* for 2-3 minutes to pellet the cells.
9. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
10. Resuspend the cells in 15 mL of T24T Expansion Medium.
11. Transfer the cell mixture to a T75 tissue culture flask.
12. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. T24T 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 T24T cells.
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 T24T 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 T24T Expansion Medium to the conical tube and resuspend the cells thoroughly.

    IMPORTANT: Do not vortex the cells.
11. Count the number of cells with a Scepter™ 3.0 handheld automated cell counter using 60 µm sensor tips.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

T24T cells may be frozen in T24T Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Nat Rev Dis Primers 2017, 3: 17022.
2. Int J Cancer 2006, 119(5): 1212-1215.
3. Genes Chromosomes Cancer 2000, 27(3): 252-263.
4. Int J Cancer 1973, 11(3): 765-773.

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