Data Sheet

HBEC30KT Human Bronchial Epithelial Cell Line

Immortalized Cell Line

SCC480

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

Bronchial epithelial cells line the airways and provide critical functions in protection from pathogens and inhaled particulates. Damage to airway epithelial cells by carcinogens such as those present in tobacco smoke is a key etiological agent of lung cancer. Non-small cell lung cancer (NSCLC) comprises 85% of lung cancers, and includes both subsets, lung adenocarcinoma and lung squamous cell carcinoma. NSCLC arises from epithelial cells in the lung, mutations in epithelial growth factor receptor (EGFR) or Kras being the most common initiators. Mutations in tumor suppressor genes TP53 and CDKN2A drive tumor progression; respectively, these genes are inactivated in 90% and 70% of non-small cell lung cancers. Although surgical resection is standard treatment in early-stage lung cancer, recurrence is common, and lung cancer remains the number one cause of cancer deaths worldwide.[1]

The HBEC30KT human bronchial epithelial cell line is a normal bronchial epithelial cell line immortalized with hTERT. HBEC30KT is useful both as normal control for non-small cell lung cancer cell lines and for knockdown/knockout modifications for investigating genetic and molecular players in tumor progression. HBEC30KT expresses normal epithelial cell markers including beta-catenin.[2]

Source

The HBEC30KT cell line was derived from normal human bronchial epithelial cells immortalized with hTERT.[2]

Short Tandem Repeat

D3S1358: 16	D13S317: 11, 12
D7S820: 8, 9	D16S539: 11
vWA: 16, 17	TH01: 9.3
FGA: 20	TPOX: 8, 9
D8S1179: 13, 14	CSF1PO: 10, 11
D21S11: 28, 30	Amelogenin: X
D18S51: 14, 16	Penta D: 9, 13
D5S818: 11	Penta E: 10

Quality Control Testing

HBEC30KT cells are verified to be of human origin and negative for mouse, rat, nonhuman primate, Chinese hamster, and Golden Syrian hamster interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.

Cells tested negative for *mycoplasma*.

Storage and Handling

HBEC30KT 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

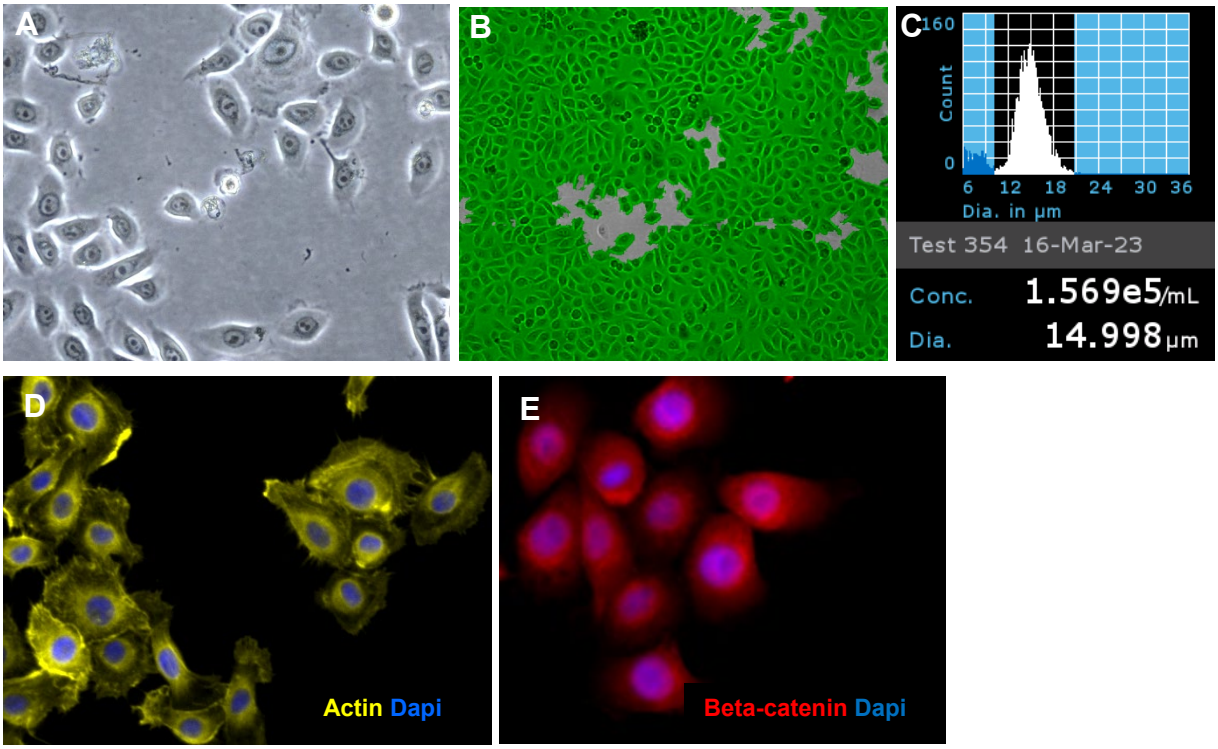

Figure 1. A. Bright-field image of HBEC30KT cells two days after thaw in a T75 flask. **B.** Cell confluency was assessed throughout the culture using the MilliCell® Digital Cell Imager (MDCI10000). **C.** Cell counting was performed using Scepter™ 3.0 Handheld Automated cell counter using 60 µm sensors (PHCC360KIT). **D.** Cells express actin (65906) and **E.** beta-catenin (ABE208).

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.
2. Cells are thawed and expanded in HBEC30KT Expansion Medium (Cell Applications human tracheal/bronchial epithelial growth medium, 511-500).
3. Remove the vial of frozen HBEC30KT 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 HBEC30KT 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 HBEC30KT 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 the Cells

1. Do not allow the cells to grow to confluency. HBEC30KT cells should be passaged at ~80-85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of HBEC30KT cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 3-5 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 5-7 mL of HBEC30KT 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 HBEC30KT 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

HBEC30KT cells may be frozen in HBEC30KT Expansion Medium supplemented with 10% FBS and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Herbst, R. S., Morgensztern, D., & Boshoff, C. (2018). The biology and management of non-small cell lung cancer. *Nature*, *553*(7689), 446-454.
2. Zaganjor, E., Osborne, J. K., Weil, L. M., Diaz-Martinez, L. A., Gonzales, J. X., Singel, S. M., ... & Cobb, M. H. (2014). Ras regulates kinesin 13 family members to control cell migration pathways in transformed human bronchial epithelial cells. *Oncogene, 33*(47), 5457-5466.

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