Data Sheet

# HCC95 Human Lung Squamous Cell Carcinoma Line

Cancer Cell Line

**SCC483**

**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

Lung cancer is a significant cause of death worldwide. Among all lung cancer cases, 85% are categorized as non-small cell lung cancer (NSCLC) which is a heterogeneous group of diseases with poor prognosis having a predicted 5-year survival rate.[1,2] Among all NSCLC cases, 30-40% are categorized as lung squamous carcinoma (LUSC) which often affects the main airways and bronchi and whose etiology is attributed to smoking.[3,4]

Among the NSCLC cell lines, HCC95 has been shown to replicate LUSC disease phenotype. Early research showed that the HCC95 line is highly similar to the original tumor genetically and histochemically—with poor differentiation and formation of keratinization.[5] Recently, transcriptomic characterizations show that HCC95 shows high similarity to the typical LUSC transcriptome.[6] Collectively, these demonstrate that HCC95 is a valuable cell line in modeling LUSC with high fidelity *in vitro*. HCC95 is often used as an *in vitro* model for NSCLC or LUSC. A 2010 study has exemplified the use of HCC95 in studying NSCLC sensitivity to cisplatin treatment.[7]

## Source

The HCC95 cell line was derived from a 65-year-old male with NSCLC.[5]

## Short Tandem Repeat

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D3S1358: | 17 | D8S1179: | 14, 16 | D13S317: | 11, 12 | CSF1PO: | 10, 12 |
| D7S820: | 10 | D21S11: | 29, 30 | D16S539: | 9 | AMEL: | X |
| vWA: | 14, 17 | D18S51: | 16, 20 | TH01: | 8, 9 | Penta D: | 9 |
| FGA: | 22 | D5S818: | 11, 12 | TPOX: | 8, 11 | Penta E: | 12, 17 |

Cancer cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozyosity of alleles at one or more genetic sites with increased passages.

## Quality Control Testing

Each vial contains ≥1 × $10^6$ 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

HCC95 cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

## Presentation

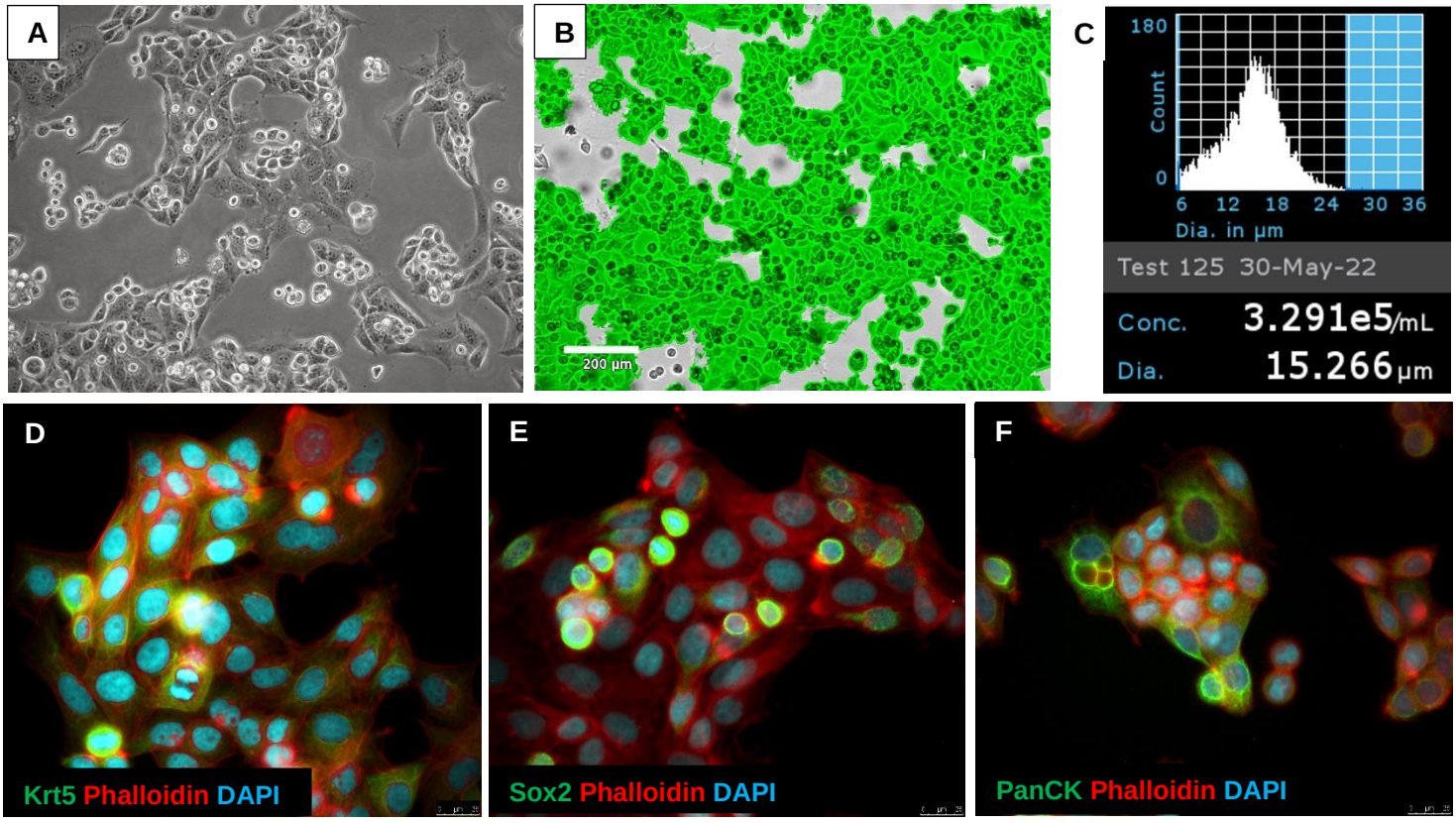


**Figure 1.** (**A**) Bright-field image of HCC95 cells three days after thaw in a T75 flask. (**B**) Cell confluency (79%) was assessed throughout the culture using MiliCell® Digital Cell Imager (MDCI 10000). (**C**) Cell counting was performed using Scepter™ 3.0 handheld automated cell counter (PHCC360KIT) using 60 µm sensor tips. (**D**) HCC95 cells express the squamous carcinoma marker, keratin 5 (Krt5), (**E**) cancer stem cell marker, Sox2, and (**F**) general epithelial marker, pan-cytokeratin (panCK).

## 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.

   Cells are thawed and expanded in HCC95 Expansion Medium comprised of RPMI-1640 Medium (R0883) with 10% FBS (ES-009-B), 2 mM L-Glutamine (TMS-002-C), 1 mM Sodium pyruvate (S8636), and 1X Penicillin/Streptomycin (TMS-AB2-C, optional).
2. Remove the vial of frozen HCC95 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.

   **Note:** 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 HCC95 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   **Note:** 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.

   **Note:** Do not vortex the cells.
7. Centrifuge the tube at 1100 rpm 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 20 mL of HCC95 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. HCC95 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 HCC95 cells. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5 mL of Accutase® or Trypsin/EDTA solution and incubate in a 37 °C incubator for 5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 8 mL of HCC95 Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 *x g* for 3-5 minutes to pellet the cells.
8. Discard the supernatant.
9. Add 2-5 mL of HCC95 Expansion Medium to the conical tube and resuspend the cells thoroughly.

   **Note:** Do not vortex the cells.
10. Count the number of cells using a hemocytometer or a Scepter 3.0 handheld automated cell counter using 60 µm sensor tips.
11. Plate the cells to the desired density. Typical split ratio is 1:4 to 1:8. The medium should be replaced every other day.

### Cryopreservation of Cells

HCC95 cells may be frozen in HCC95 Expansion Medium and 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

## References


1. J Natl Compr Canc Netw. 2013, 11(6): 645–653 DOI: 10.6004/jnccn.2013.0084
2. Nat Rev Cancer 2014, 14(8): 535–546 DOI: 10.1038/nrc3775
3. J Thorac Dis. 2013, 5(Suppl 5): S463–S478 DOI: 10.3978/j.issn.2072-1439.2013.08.43
4. J Clin Oncol. 2010, 28(36): 5311–5320 DOI: 10.1200/JCO.2010.28.8126
5. Clin Can Res. 1999, 5(5): 991-1000
6. Nat Commun. 2019, 10(1): 3574 DOI: 10.1038/s41467-019-11415-2
7. J Cancer Res Clin Oncol 2010, 136(4): 493–502 DOI: 10.1007/s00432-009-0681-6


## Academic Use Agreement

Subject to local law

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means HCC95 Human Lung Squamous Carcinoma Cell Line (SCC483).

"Academic Research" means any internal in vitro research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

## Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

### Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

### Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

### Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.



Document Template 20306518 Ver 6.0
23200332 Ver 1.0, Rev 18Mar2025, UD