

Data Sheet

# HSC-3 Human Tongue Squamous Carcinoma Cell Line

Cancer Cell Line

**SCC193****Pack size:  $\geq 1 \times 10^6$** **Store in Liquid Nitrogen****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for Human or Animal Consumption.**

## Background

Squamous cell carcinoma is the most common malignant neoplasm found in the oral cavity and cervical metastases are frequently observed.<sup>1</sup> Suppression of regional lymph node metastases is one of the most significant factors affecting favorable prognosis of squamous cell carcinoma.<sup>1</sup> Experimental models of lymphogenous metastasis analogous to progression of human oral carcinoma are needed to provide an opportunity to investigate metastatic mechanisms.

Athymic nude mice xenografted with human carcinoma have been used as an *in vivo* model of tumor invasion and metastasis. Most reports indicate that metastasis of human epithelial tumors inoculated subcutaneously into nude mice is rather rare. HSC-3 is a human oral squamous carcinoma cell line that forms metastatic foci in the draining lymph nodes when subcutaneously transplanted into nude mice.<sup>2</sup> Conditioned media from HSC-3 has been shown to promote angiogenesis, HSC-3 cells having markedly elevated levels of growth factor secretion.<sup>1</sup> The highly aggressive characteristics of this cell line have made HSC-3 a highly cited model for the study of metastatic squamous cell carcinoma.

## Source

The HSC-3 cell line was established from tumors of metastatic lymph nodes originated in tongue squamous cell carcinomas of a 63-year-old male patient. Epithelial cells that migrated and proliferated were collected and subcultured from fresh explanted tumor tissues.<sup>2</sup>

## Short Tandem Repeat

D3S1358: 17	D16S539: 9	Penta E: 11, 15	D8S1179: 12
TH01: 6, 9.3	CSF1PO: 11	D5S818: 11, 13	TPOX: 8
D21S11: 30, 31.2	Penta D: 10	D13S317: 12	FGA: 22, 26
D18S51: 15	vWA: 14, 17	D7S820: 13	Amelogenin: X, Y

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

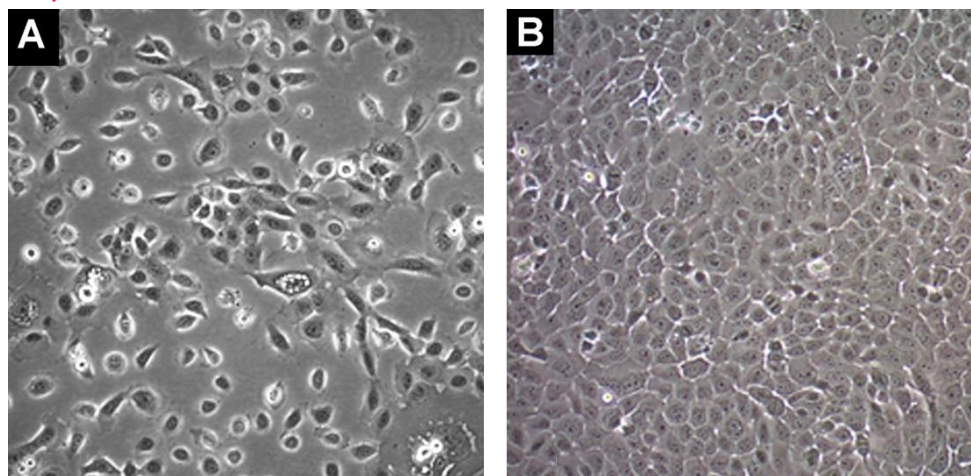
## Quality Control Testing

- Each vial contains  $\geq 1 \times 10^6$  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 rat, mouse, 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

HSC-3 human tongue squamous carcinoma cell line should be stored in liquid nitrogen. 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.** HSC-3 cells one (A, 10X magnification) and two (B, 10X magnification) days after thawing in a T75 flask.

## 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.  
HSC-3 Expansion Medium: Cells are thawed and expanded in DMEM-High Glucose (SLM-120-B) supplemented 10% FBS (ES-009-B).
2. Remove the vial of frozen HSC-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 HSC-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. Check cell suspension under microscope to ensure no cell clumps are present. If cell clumps are present, perform step 9 after steps 7 and 8.  
**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. If clumps of cells are present, dissociate cells with 5 mL trypsin-EDTA or Accutase® in 37 °C incubator for 5-15 minutes, checking every 5 minutes for dissociation. After dissociation is complete, if using trypsin inactivate trypsin with 5 mL 10% FBS medium. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
10. Resuspend the cells in 15 mL of HSC-3 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<sub>2</sub>.

### Subculturing Cells

1. Do not allow the cells to grow to confluency. HSC-3 cells should be passaged at ~80-85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the HSC-3 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. If clumps of cells are present, incubate an additional 5-10 minutes in 37 °C incubator, checking every 5 minutes for dissociation of cells.
6. Add 5-7 mL of HSC-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 HSC-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

HSC-3 human tongue squamous cell carcinoma cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container. Ensure cells are completely dissociated prior to cryopreservation.

### References

1. Michi Y, Morita I, Amagasa T, Murota S (2000) Oral Oncol 36(1): 81-88.
2. Momose F et al., (1989) J Oral Pathol Med 18(7): 391-395.

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