

**SiHa Cells | 305023**

**Description**

SiHa cells are a human cervical squamous cell carcinoma cell line that has been widely used in research for several decades. They were isolated from primary uterine biopsy fragments from a 55-year-old female Japanese patient with squamous cell carcinoma. This cell line is of great interest to researchers studying cervical cancer and other related diseases due to their unique genetic characteristics.

SiHa cells have been found to express the p53+ and pRB+ genes, which are involved in cell cycle regulation, DNA repair, and tumor suppression. These genes make SiHa cells an ideal model for studying the molecular mechanisms of cancer development and progression. Additionally, SiHa cells are a suitable transfection host, making them an excellent tool for gene expression studies.

SiHa cells have a hypertriploid karyotype, with an average chromosome number between 69 and 72. The SiHa cells are HPV-16 positive, showing integration of 1 to 2 copies of the viral genome per cell. Cells are tumorigenic, forming poorly differentiated epidermoid carcinoma (grade III) in nude mice. This makes them an excellent model for studying cancer progression and testing anti-cancer drugs.

SiHa cell line expresses various isoenzymes, including AK-1, ES-D, G6PD, GLO-I, Me-2, PGM1, and PGM3. Electron microscopy revealed abundant tonofilaments in the cytoplasm and desmosomes at the cell junctions. The growth properties of SiHa cells are adherent, with a doubling time of 17 hours in 10% FBS media and 21 hours in 5% FBS media. Epithelial cell adhesion molecule (EpCAM) expression is present in 92% of SiHa cells, indicating their epithelial origin. They show strong cytokeratin expression but no vimentin expression.

**Organism** Human

**Tissue** Cervix

**Disease** Human papillomavirus-related cervical squamous cell carcinoma

**Synonyms** Siha, SIHA

**Age** 55 years

**Gender** Female

**Ethnicity** Asian

**Morphology** Epithelial

**Growth properties** Adherent

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**Citation** SiHa (Cytion catalog number 305023)

**Biosafety level** 1

**NCBI\_TaxID** 9606

**CellosaurusAccession** CVCL\_0032

**Tumorigenic** Yes

**Culture Medium** EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO<sub>3</sub>, w: EBSS (Cytion article number 820100a)

**Supplements** Supplement the medium with 10% FBS and 1% NEAA

**Dissociation Reagent** Accutase

**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

**Fluid renewal** 2 to 3 times per week

**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.