

## CAL-33 Cells | 305496

### Description

The CAL-33 cell line is a human squamous cell carcinoma line derived from a primary tumor of the tongue. Established from a male patient with moderately differentiated squamous cell carcinoma, CAL-33 cells are known for their robust growth in vitro and tumorigenic capacity when injected into immunocompromised mice. These cells display polygonal epithelial morphology, with a doubling time of approximately 43 hours. Given its origin, CAL-33 serves as an effective model for studying oral and head and neck squamous cell carcinoma (HNSCC) biology, specifically in contexts where HPV-negative carcinoma models are necessary.

CAL-33 is particularly valuable in radiation oncology research due to its well-characterized subclones with varying degrees of radioresistance and radiosensitivity. Studies on these subclones have shown distinct genomic and transcriptomic profiles, which contribute to differential radiation responses. Pathways associated with radioresistance in CAL-33 include DNA repair, senescence, apoptosis, and PI3K/AKT signaling, with additional involvement of genes linked to the senescence-associated secretory phenotype (SASP). These features make CAL-33 a significant tool for investigating radiation-induced cellular responses and identifying potential therapeutic targets aimed at overcoming radioresistance in HNSCC.

Moreover, the CAL-33 cell line is also used for drug sensitivity studies, as it exhibits sensitivity to various chemotherapeutic agents. This versatility in applications-ranging from basic oncogenic pathway elucidation to applied therapeutics and radiation studies-has solidified CAL-33 as a prominent cell line in cancer research focused on aggressive squamous cell carcinomas of the oral cavity.

**Organism** Human

**Tissue** Tongue

**Disease** Squamous cell carcinoma

**Synonyms** Cal-33, CAL 33, CAL33, CAL-SCC-33, Centre Antoine Lacassagne-33

**Age** 69 years

**Gender** Male

**Ethnicity** Caucasian

**Morphology** Epithelial-like

**Growth properties** Adherent, monolayer

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**Citation** CAL33 (Cytion catalog number 305496)

**Biosafety level** 1

**NCBI\_TaxID** 9606

**CellosaurusAccession** CVCL\_1108

**Mutational profile** Mutation: Tmprss2, p.Gly8Val (c.23G>T) (c.-57+99G>T), homozygous; Mutation: TP53, p.Arg175His (c.524G>A)

**Culture Medium** DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO<sub>3</sub>, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)

**Supplements** Supplement the medium with 10% FBS

**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.

**Seeding density** 1 - 2 x 10<sup>4</sup> cells/cm<sup>2</sup>

**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.