

**C4-2 Cells | 305752**

**Description**

The C4-2 cell line is an androgen-independent human prostate cancer model derived from the parental LNCaP cell line. It was established through a stepwise in vivo selection process involving co-injection of LNCaP cells with human bone stromal cells (MS cells) into castrated immunodeficient mice, leading to the emergence of androgen-insensitive tumors. The C4-2 subline was specifically derived from the C4 variant after further passage in castrated hosts, and it retains the ability to grow and form tumors in androgen-depleted conditions without the need for stromal support.

C4-2 cells maintain the prostate-specific antigen (PSA) production and expression of the androgen receptor (AR), including the characteristic T877A AR point mutation inherited from LNCaP, but display reduced androgen responsiveness compared to the parental line. While LNCaP cells require androgens for growth, C4-2 cells proliferate in androgen-depleted environments and continue to express PSA and AR-regulated genes, making them a robust model for castration-resistant prostate cancer (CRPC). In vitro, C4-2 cells grow more rapidly than LNCaP under standard culture conditions, and they also exhibit improved tumorigenicity in vivo. When injected subcutaneously into immunocompromised mice, C4-2 cells readily form tumors, a feature that contrasts with the slower or less consistent tumorigenic potential of LNCaP cells.

The C4-2 model has been widely used to study mechanisms of resistance to androgen deprivation therapy (ADT), the role of intracrine androgen metabolism, and the molecular pathways underpinning CRPC progression. It retains expression of prostate-specific membrane antigen (PSMA), although at lower levels than LNCaP, and displays unique responses to androgen stimulation and antiandrogen therapies. These attributes make C4-2 a cornerstone model for evaluating new therapeutics targeting advanced prostate cancer.

**Organism**

Human

**Tissue**

Metastatic

**Disease**

Prostate carcinoma

**Synonyms**

LNCaP-C4-2, LNCaP subline C4-2, C4-2, C42, Sp 2817

**Age**

50 years

**Gender**

Male

**Ethnicity**

Caucasian

**Morphology**

Epithelial-like

**Growth properties**

Adherent

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**Citation** C4-2 (Cytion catalog number 305752)

**Biosafety level** 1

**NCBI\_TaxID** 9606

**CellosaurusAccession** CVCL\_4782

**Mutational profile** Mutation: AR, Simple, p.Thr878Ala (c.2632A>G), Hemizygous. Mutation, MEN1, Simple, p.Tyr318Ter (c.954T>G) (p.Tyr313Ter, c.939T>A), Heterozygous (from parent cell line). Mutation, PIK3R1, Simple, p.Arg639Ter (c.1915C>T), Heterozygous (from parent cell line). Mutation, PTEN, Simple, p.Lys6Argfs\*4 (c.17\_18delAA), Unspecified (from parent cell line).

**Seeding density** 2 - 3 x 10<sup>4</sup> cells/cm<sup>2</sup>

**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.