Product Sheet
C4-2 (ATCC® CRL-3314™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level 1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is 400 mL DMEM (Lonza cat# 12-741) plus 100 mL F12 Medium (Lonza cat# 12-615F). To make the complete medium add the following components:
- 10% Fetal Bovine Serum (ATCC 30-2020; heat-inactivate before using)
- 5.6 mL T-medium supplement
To prepare the T-medium supplement, dilute the following components into a 0.1% BSA in PBS (ATCC 30-2200) solution: 50 mg Insulin (Gibco cat# 12585-014), 10 µg/ml final concentration; 136 ng Triodo-L-thyronine (Sigma cat# T2877), 13.6 µg/ml final concentration; 50 µg Transferrin (Sigma cat# T4382), 5 µg/ml final concentration; 2.5 mg D-Biotin (Sigma cat# 47868), 0.25 µg/ml final concentration; and 250 µg Adenine (Sigma cat# A3159), 25 µg/ml final concentration
Combine all components, mix well, filter sterilize the T-medium supplement using a 0.22-micron filter. Aseptically dispense 5.7 mL into sterile tubes.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: C4-2 (ATCC® CRL-3314™)

Organism: Homo sapiens, human
Tissue: prostate
Disease: prostate cancer
Gender: male
Morphology: epithelial-like
Growth Properties: adherent

DNA Profile:
- Amelogenin: X,Y
- CSF1PO: 9,10,11
- D13S317: 10,11
- D16S539: 10,11
- D7S820: 9.1,10.3
- THO1: 9
- TPOX: 8,9
- vWA: 16,18

SAFETY PRECAUTION

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium, and spin at approximately 150-400 x g for 8 to 12 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Dilute 1:5 - 0.05% Trypsin (ATCC catalog # PCS-999-003) in PBS
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Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.

Cultures can be established between 3 x 10⁴ and 6 x 10⁴ viable cells/cm².

Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 3 x 10⁴ and 1 x 10⁵ cells/cm².

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week

Cryopreservation Medium

Culture medium + 5% DMSO

Comments

The human prostatic carcinoma cell line, LNCaP (1 x 10⁶ cells; passage # 29) as described in Horoszewiez, JS et al. Cancer Research 43:1809-1818, 1983; was co-inoculated into an athymic male nude mouse with (1 x 10⁵) human fibroblasts derived from an osteosarcoma (cell line MS). The nude mouse host was castrated after 8 weeks incubation. A tumor specimen was excised after a total of 12 weeks. The C4 cell line constitutes the in vitro cultured subline grown from the murine host’s tumor. When the C4 sub-line was subsequently co-inoculated with MS osteosarcoma fibroblasts in a castrated athymic male nude mouse host for another 12 weeks by the same protocol described above. Prostatic epithelial cells cultured from the resultant tumor in this host constituted the C4-2 subline. Tumorigenicity & Osseous Metastasis: Orthotopic administration of 1 x 10⁵ resuspended C4-2 cells in both intact and castrated athymic male nude mice yielded 100% tumorigenicity (20/20 and 14/14, respectively). Osseous prostate cancer metastases were detected in both intact and castrated murine hosts (2/20 and 3/14, respectively).

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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