C4-2 (ATCC® CRL-3314™)

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Descriptive Information

**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
- D5S818: 11,12
- D7S820: 9.1,10.3
- TH01: 9
- TPOX: 8.9
- vWFA: 16,18

**Batch-Specific Information**

Refer to the Certificate of Analysis for batch-specific test results.

**SAFETY PRECAUTION**

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

**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 DPBS (ATCC 30-2200) to remove all traces of serum that contains
Add 2.0 to 3.0 mL of Trypsin-EDTA (ATCC 30-2101) 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.

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

5. Add appropriate aliquots of the cell suspension to new culture vessels.

6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 1.5 X 10^6 and 3.1 X 10^6 cell/cm².

Subcultivation Ratio: A subcultivation ratio of 1:8 to 1:10 is recommended

Medium Renewal: 2 to 3 times per week

Cytotoxicity

The complete growth medium is cytotoxic to most normal human cells when used at high concentrations (50% or more) for prolonged periods (24 hours or more) at 37°C.

Complete Growth Medium

The base medium for this cell line is DMEM/F12(4:1). To make the complete medium add: 10% FBS (ATCC 30-2020), heated inactivated; 0.100 μg/mL Insulin; 275 ng/mL Triiodothyronine; 88.6 ng/mL apo-Transferrin; 4.9 ng/mL d-Biotin; 251.8 ng/mL Adenine. Combine all components, mix well, and sterilize using a 0.22-micron filter. Aseptically dispense 5.7 mL into sterile tubes. Store at -20°C.

Cryopreservation Medium

95% complete growth media + 5% DMSO (ATCC 4-X). Store at liquid nitrogen vapor phase.

Comments

The human prostatic carcinoma cell line, LNCaP (1 x 10⁶ cells; passage # 29) as described in Horoszewicz, JS et al. Cancer Research 43:1809-1818, 1983; was co-inoculated into an athymic male nude mouse with (1 x 10⁶) 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 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 10⁶ 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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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™)

The C4 cell line...
Please read this FIRST

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Intended Use

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

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