**C4-2 (ATCC® CRL-3314™)**

**Product Sheet**

**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 Triiodo-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™)

**Description**

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

**DNA Profile:**
- Amelogenin: X,Y
- CSF1PO: 9,10,11
- D13S317: 10,11
- D16S539: 10,11
- DSS518: 11,12
- D7S820: 9,10,13
- THO1: 9
- TPOX: 8,9
- vWA: 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 Dilute 1:5 - 0.05% Trypsin (ATCC catalog # PCS-999-003) in PBS.
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.

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

Cultures can be established between 3x $10^4$ and 6 x $10^4$ viable cells/cm².

6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 3 x $10^4$ and 1 x $10^5$ 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 $10^6$ 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 $10^2$) 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: Orthotropic administration of 1 x $10^5$ 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](http://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.

**ATCC Warranty**

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

**Disclaimers**

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this
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 Triiodo-L-thyronine (Sigma cat# T2877), 13.6 pg/ml final concentration; 50 mg 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 mg 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™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor