



UACC-732

CRL-3166™

Description

Organism: *Homo sapiens*, human

Tissue: Breast

Age: 33 years

Gender: Female

Morphology: epithelial-like

Growth properties: Adherent

Disease: Inflammatory Carcinoma

Storage Conditions

Product format: Frozen

Storage conditions: Vapor phase of liquid nitrogen

Intended Use

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

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies

and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C

Atmosphere: 100% Air

Handling Procedures

Unpacking and 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.

Complete medium:

The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 5%
- 0.01 mg/ml transferrin (final conc.)
- 0.01 mg/ml insulin (final conc.)
- 5 µg/mL (55 U/ml) catalase (final conc.)
- 3.6 µg/mL (0.01 mM) hydrocortisone (final conc.)
- 70 µg/mL (0.5mM) o-phosphoethanolamine (final conc.)
- 10 ng/ml human recombinant epidermal growth factor (EGF) (final conc.)
- 3 ng/ml (0.01 µM) estradiol (final conc.)
- 0.8 ng/ml (1 pM) Na-L-thyroxine (final conc.)
- 23 µg/mL (0.2mM) proline (final conc.)
- extra 2 mM glutamine

Note: Do not filter complete medium.

L-15 Medium is formulated for use in a free gas exchange with atmospheric air. A CO₂ and air mixture is detrimental to cells when using this medium for cultivation.

Handling Procedure:

Note: These cells are cultured on collagen I coated vessels. Please see Subculturing Procedure for details.

To insure 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. It is recommended that the cryoprotective agent be removed immediately.

Centrifuge the cell suspension at approximately 125 x *g* for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.

4. Transfer the cell pellet to an appropriate size vessel. 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 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).
5. Incubate the culture at 37°C in a suitable incubator. A 100% air atmosphere is recommended if using the medium described on this product sheet. **Note: This cell line grows slowly.**

Subculturing procedure:

These cells are cultured on collagen I coated vessels. Add 5 µg per cm² collagen I (BD Biosciences, Cat. No.354236 or equivalent) to culture vessels and incubate at room temperature for 1 hour. Remove collagen solution and rinse vessels 3 times with a balanced salt solution. Vessels may be used immediately or air dried and stored at 2-8°C for up to one week under sterile conditions. Alternatively, commercially available pre-coated Collagen I vessels, such as BD BioCoat Cellware (BD Biosciences, Cat. No. 356524 for 75 cm² flask) or equivalent, may be used.

Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of solutions for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (DPBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. 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.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x *g* for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new collagen I coated culture vessels. An inoculum of 3 X

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10^4 to 4×10^4 viable cells/cm² is recommended.

7. Incubate cultures at 37.0°C.

Subculture when cultures reach a cell concentration between 7×10^4 to 1×10^5

Subcultivation ratio: A subcultivation ratio of 1:2 is recommended.

Medium renewal: every 2 to 3 days

Note: This cell line grows slowly.

Reagents for cryopreservation: Complete growth medium supplemented with 10% (v/v) fetal bovine serum and 10% DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: UACC-732 (ATCC CRL-3166)

References

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

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Revision

This information on this document was last updated on 2023-01-01

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
