



# COALA

CRL-3647™

## Description

COALA are human adherent colorectal cancer cells that can grow in 2D and 3D cultures. In 3D culture such as Matrigel, COALA cells form circle-shaped spheroids. They can recapitulate the in vivo structure of tumors and are suitable for drug development.

**Organism:** *Homo sapiens*, human

**Cell Type:** epithelial cell

**Tissue:** Colon

**Gender:** Male

**Morphology:** Epithelial-like

**Growth properties:** Adherent

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## 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.

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## 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.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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## Handling Procedures

**Complete medium:** The base medium for this cell line is DMEM/F-12, GlutaMAX™ (ThermoFisher Scientific catalog # 10565018) plus fetal bovine serum (ATCC catalog # 30-2020) to a final concentration of 10%.

Culture medium is supplemented with 10  $\mu$ M ROCK Inhibitor (ATCC ACS-3030) at start-up procedure only.

**Handling Procedure:** 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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

1. Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  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 to 400 x g for 8 to 12 minutes.
4. Carefully aspirate the supernatant and discard, leaving the cell pellet.
5. Gently resuspend the cell pellet with the appropriate amount of complete growth medium containing ROCK inhibitor and transfer cell suspension into a vented T-75.
6. Place the flask in a  $37^{\circ}\text{C}$  incubator with 5%  $\text{CO}_2$ .

**Subculturing procedure:**

Volumes used in this protocol are for 75  $\text{cm}^2$  flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning<sup>®</sup>

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of 0.25% Trypsin/0.53 mM EDTA (ATCC 30-2101™) to flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 minutes) Detaching of COALA at higher confluency can take 10-15 minutes.  
Cells that are difficult to detach may be placed at  $37^{\circ}\text{C}$  to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium aspirate cells by gently pipetting.
5. Centrifuge the cell suspension at approximately 150 to 400 x g for 8 to 12 minutes to remove dissociation agent.
6. Resuspend the cell pellet in an appropriate amount of complete culture

medium

7. Add appropriate aliquots of the cell suspension to new culture vessels.
8. Cultures can be established between  $4.0 \times 10^4$  and  $7.0 \times 10^4$  viable cells/cm<sup>2</sup>.
9. Incubate cultures at 37°C.

**Interval:** Seed cells at subculture at a cell concentration between  $3.0 \times 10^4$  and  $5.0 \times 10^4$  cell/cm<sup>2</sup>.

**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended

**Medium Renewal:** Every 2 to 3 days

**Note:** Cells loosely attach after seeding with heavy floating cells. Cells grow in clusters making colonies and may not reach 100% confluence. Subculture should be performed if dark and dense spot in clusters is observed.

**Reagents for cryopreservation:** Complete culture medium + 10% DMSO (ATCC 4-X)

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: COALA (ATCC CRL-3647)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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