



FHC

CRL-1831™

Description

FHC is an adherent cell line isolated from the large intestine of a healthy donor. This cell line is non-malignant and can be used to study the development and functions of the human colon in gastrointestinal (GI) research. FHC can also be used for pharmacologic and toxicologic studies on colonic epithelial cells.

Organism: *Homo sapiens*, human

Cell Type: colonocyte

Tissue: Large intestine; Colon

Age: 13 weeks gestation

Morphology: epithelial

Growth properties: Adherent

Disease: Normal

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: 95% Air, 5% CO₂

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 DMEM:F12 Medium (ATCC 30-2006). To make the complete growth medium, add the following components to the base medium:

- extra 10 mM HEPES (for a final conc. of 25 mM)
- 10 ng/ml cholera toxin
- 0.005 mg/ml insulin
- 0.005 mg/ml transferrin
- 100 ng/ml hydrocortisone
- 20 ng/mL human recombinant EGF (Thermo Fisher PHG0311)
- Fetal Bovine Serum, 10% final conc (ATCC 30-2020)

Example media preparation:

500 mL DMEM:F'12 (ATCC 30-2006)

56 mL FBS (ATCC 30-2020) - not heat-inactivated

5.7 mL Gibco HEPES (ThermoFisher Scientific Catalog # 15630-080)

0.71 mL Gibco Human Insulin (ThermoFisher Scientific Catalog # 12585-014)

1.6 mL Hydrocortisone, 36 ug/mL stock (Sigma Catalog # H-0135)

56 uL Cholera Toxin, 100 pg/mL stock (Sigma Catalog # C8052)

Note: The above prepared media is stable for 30 days stored at 2.0 to 8.0°C.

Note: Due to limited stability, the following components should be added to an aliquot of the above culture medium fresh prior to seeding or performing full fluid change. Complete culture medium with the below components is stored at 2.0 to 8.0°C and expires in 7 days.

Transferrin, 1 mg/mL stock (Sigma catalog # T1147): use 5uL/1 mL culture medium

EGF Stock (11.2 ug/mL EGF - see preparation notes below): use 2 uL/1 mL culture medium

To prepare 11.2 ug/mL EGF stock, aseptically combine:

- 100 ug Gibco EGF (ThermoFisher Scientific catalog # PHG0311)
- 8.9 mL PBS with 0.1% BSA

Store in working aliquots at -20°C. EGF is stable for 1 year when prepared and stored as directed. Do not repeat freeze/thaw.

Handling Procedure:

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. 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 (200 x *g* for 8 minutes).
4. Resuspend cell pellet with the recommended complete growth medium (see the lot information on Certificate of Analysis (COA) for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² culture flask as recommended on the COA. **Note:** For this cell line a T25 flask is recommended because of the number of cells provided. The recommended seeding density for CRL-1831 is 6.0×10^3 to 1.0×10^4 viable cells/cm². 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).
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.

Additional notes for culturing:

CRL-1831 is an extremely slow-growing cell line. The cells are large and tend to be granular and irregular shaped.

When the cells are seeded into new flasks, they tend to be large and spread out. As the cells begin to divide and grow, the newly divided cells are smaller and more epithelial-like. The new growth tends to appear in patches which gradually become more and more confluent.

Subculturing procedure:

Volumes are given for a 75 cm² flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that 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. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x *g* for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum of 4 to 6 X 10³ viable cell/cm² is recommended.
6. Incubate cultures at 37°C.

Note: Unless floating cells have been verified to be non-viable via a trypan blue or some other sort of viability assay, floating cells should be kept with the main culture.

Subcultivation Ratio/Subculture seeding density: A subcultivation ratio of 1:2 to 1:4 or or subculture at a seeding density of 4.0 x 10³ to 6.0 x 10³ viable cells/cm² is recommended every 10 to 15 days

Medium Renewal: Every 3 to 4 days.

Reagents for cryopreservation: DMEM:F12 Medium supplemented with 10 mM HEPES, 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 100 ng/ml hydrocortisone, 20 ng/ml human recombinant EGF, 7% FBS. Lots produced prior to June 2020 may have used a different cryopreservation medium (complete growth medium supplemented with an extra 50% FBS and 10% (v/v) DMSO), contact our Product Experience Specialists for further details.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: FHC (ATCC CRL-1831)

References

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

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