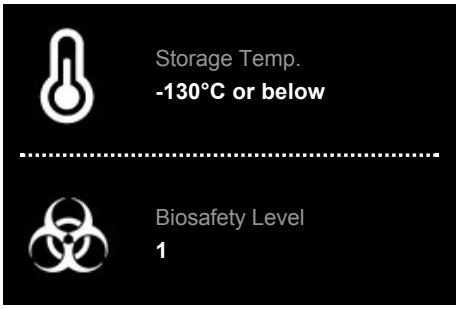
ATCC

Product Sheet

Primary Dermal Fibroblasts; Normal, Human, Neonatal, Mitomycin C Treated (ATCC® PCS-201-011™)

Please read this FIRST



Intended Use

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

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Primary Dermal Fibroblasts; Normal, Human, Neonatal, Mitomycin C Treated (ATCC® PCS-201-011™)

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

Description

ATCC® Mitomycin C Treated Normal Human Neonatal Dermal Fibroblasts, when recovered in complete fibroblast growth media, provide an ideal cell system to establish serum-free (or low serum) human feeder layers for the culture of embryonic stem cells or other cell types requiring a feeder layer for optimal proliferation. The cells are cryopreserved in the second passage to ensure the highest viability and plating efficiency. ATCC® Primary Cell Solutions™ cells, media, supplements and reagents are quality tested together to guarantee optimum performance and reliability.

Components: One vial of Mitomycin C Treated Dermal Fibroblasts, Normal, Human Neonatal (ATCC®PCS-201-011™) containing a minimum of 3 x 10^6 viable cells (provided).

Also Required:

A. One bottle of Fibroblast Basal Medium (ATCC PCS-201- 030) plus one Fibroblast Growth Kit of either:
   1. Fibroblast Growth Kit - Serum-Free (ATCC PCS- 201-040) containing each of the following growth supplements: L-glutamine, hydrocortisone hemisuccinate, HLL supplement (human serum albumin, linoleic acid, lecithin), rh FGF-beta, rh EGF / TGF beta-1 supplement, rh insulin and ascorbic acid.
   2. Fibroblast Growth Kit - Low Serum (ATCC PCS- 201-041) containing each of the following growth supplements: L-glutamine, hydrocortisone hemisuccinate, rh FGF-beta rh insulin, ascorbic acid and Fetal Bovine Serum.

B. Optional media supplements
   1. Gentamicin-Amphotericin B Solution (ATCC PCS-999-025)
   2. Penicillin-Streptomycin-Amphotericin B Solution (ATCC PCS-999-002)
   3. Phenol Red (ATCC PCS-999-001)

Cell Characteristics

Tissue: Foreskin

Morphology: Spindle-shaped; cells are bipolar and refractile.

Growth Properties: Adherent; mitotically arrested.

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 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 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.

Preparation of Complete Growth Medium

1. Obtain one growth kit from the freezer; make sure that the caps of all containers are tight.
2. Thaw the components of the growth kit just prior to adding them to the basal medium. It is necessary to warm the L-glutamine component in a 37°C water bath, and shake to dissolve any precipitates prior to adding to the basal medium.
3. Obtain one bottle of Fibroblast Basal Medium (480 mL) from cold storage.
4. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying them with 70% ethanol.
5. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, transfer the volume of each growth kit component, as indicated in either Table 1 or 2, to the bottle of basal medium using a

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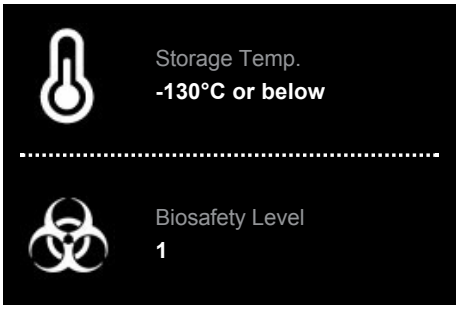

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separate sterile pipette for each transfer.

6. Tightly cap the bottle of complete growth medium and swirl the contents gently to assure a homogeneous solution. Do not shake forcefully to avoid foaming. Label and date the bottle.
7. Complete growth media should be stored in the dark at 2°C to 8°C (do not freeze). When stored under these conditions, complete growth media is stable for 30 days.

Table 1. If using the Fibroblast Growth Kit–Serum-Free (ATCC PCS-201-040), add the indicated volume for each component in the order shown.

Component	Volume	Final Concentration
L-glutamine	18.75 mL	7.5 mM
Hydrocortisone Hemisuccinate	0.5 mL	1 µg/mL
HLL Supplement	1.25 mL	HSA 500 µg/mL Linoleic Acid 0.6 µM Lecithin 0.6 µg/mL
rh FGF b	0.5 mL	5 ng/mL
rh EGF / TGF b-1 Supplement	0.5 mL	5 ng/mL 30 pg/mL
rh Insulin	0.5 mL	5 µg/mL
Ascorbic acid	0.5 mL	50 µg/mL

Table 2. If using the Fibroblast Growth Kit–Low Serum (ATCC PCS-201-041), add the indicated volume for each of the following components:

Component	Volume	Final Concentration
rh FGF b	0.5 mL	5 ng/mL
L-glutamine	18.75 mL	7.5 mM
Ascorbic acid	0.5 mL	50 µg/mL
Hydrocortisone Hemisuccinate	0.5 mL	1 µg/mL
rh Insulin	0.5 mL	5 µg/mL
Fetal Bovine Serum	10.0 mL	2%

Antimicrobials and phenol red are not required for proliferation, but may be added if desired. The recommended volume of each optional component to be added to the complete growth media is summarized in Table 3.

Table 3. Addition of Antimicrobials/Antimycotics and Phenol Red (Optional)

Component	Volume	Final Concentration
Gentamicin-Amphotericin B Solution	0.5 mL	Gentamicin: 10 µg/mL Amphotericin B: 0.25 µg/mL
Penicillin-Streptomycin-Amphotericin B Solution	0.5 mL	Penicillin: 10 Units/mL Streptomycin: 10 µg/mL Amphotericin B: 25 ng/mL
Phenol Red	0.5 mL	33 µM

Maintenance

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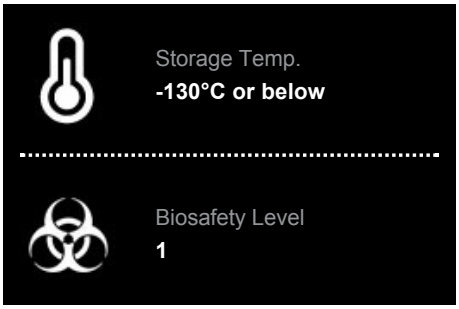

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Preparing Gelatin-Coated Tissue Culture Flasks

For optimal cell attachment, culture flasks should be prepared before thawing the cells. Non-coated tissue culture flasks can also be used.

1. Remove 0.1 % Gelatin Solution from the freezer. Allow to completely liquefy at 37°C.
2. Decontaminate the external surface of the bottle by spraying with 70 % ethanol.
3. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, add 2.5 mL of 0.1 % Gelatin Solution per 25 cm^2 of surface area to the culture flask.
4. Rock flask to coat surface; place in a 37°C incubator for at least 30 minutes, and up to overnight.
5. Aspirate the excess gelatin solution from the culture flasks using sterile technique.
6. Add 5.0 mL of complete fibroblast growth medium per 25 cm^2 of culture surface area.
7. Place the gelatin-coated flasks in a 37°C, 5% CO_2 incubator for at least one hour to equilibrate before inoculating with mitomycin C treated fibroblasts.

Handling Procedure for Frozen Cells and Establishing Feeder Layer Cultures

Before beginning, pre-warm the complete growth media in a 37°C water bath. This will take between 10 and 30 minutes, depending on the volume. If only using a small volume of medium (50 mL or less), warm only the volume needed in a sterile conical tube. Avoid warming complete media multiple times.

1. Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC PCS-201-011.
2. Using the total number of viable cells reported, determine how much surface area can be inoculated to achieve an initial seeding density of 20,000 to 40,000 cells per cm^2.
   Note: This seeding density is recommended to produce a confluent monolayer for use with stem cells. If the feeder cells are going to be used as support for another cell type (e.g., hybridoma fusions), then the seeding density should be adjusted accordingly.
3. Prepare the desired combination of gelatin-coated flasks as described in steps 1 to 7 of Preparing Gelatin-Coated Tissue Culture Flasks. If using non-coated tissue culture flasks, add 5 mL of complete growth medium per 25 cm^2 of surface area. Place the flasks in a 37°C, 5 % CO_2 incubator and allow the media to pre-equilibrate to temperature and pH for 30 minutes prior to adding cells.
4. After the culture flasks equilibrate, remove one vial of ATCC PCS-201-011 from storage and thaw the cells 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 1 to 2 minutes).
5. 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 operations from this point onward should be carried out under strict aseptic conditions.
6. Transfer the cells with a sterile pipette to a 15 mL conical tube. Add 1 mL pre-warmed complete fibroblast growth media to the cells and slowly mix. Slowly add the rest of the media to the cells in 1 mL increments. [Total volume = (1 mL x number of flasks to be seeded) - 1 mL]. These cells are very delicate and subject to osmotic shock. Gently pipette the cells to homogenize the suspension. Do not centrifuge.
7. Transfer 1.0 mL of the cell suspension to each of the pre-equilibrated culture flasks. Inoculate the cells directly onto the growth surface, cap the flasks, and gently swirl to ensure even distribution of the cells.
8. Place the seeded culture flasks in the incubator at 37°C, 5% CO_2. Flasks should remain undisturbed until the cells have attached and flattened. This will take a minimum of four hours. Change the media after 24 hours.
9. Stem cells can usually be added 24 to 48 hours after the cultures have been established.

Inoculation of the Feeder Layers with the Cells of Interest

1. Aspirate the complete fibroblast growth medium from the feeder cell layer.
2. Add pre-warmed medium appropriate for the cell type of interest (e.g., embryonic stem cell).
3. Return the culture flask to the incubator at 37°C, 5% CO_2. Allow to equilibrate for at least 30 minutes before seeding the cells of interest.

Quality Control Specifications

Growth

Each lot of ATCC® PCS-201-011 is tested to ensure that the cells support the non-differentiated growth of human embryonic stem cells in a serum-free environment and that the cells do not proliferate when maintained in complete low serum fibroblast growth media

Viability: ≥ 50% when thawed from cryopreservation.

Sterility Testing

Bacteria and Yeasts: Negative.

Mycoplasma: Negative

Viral Testing

HIV: Negative

Hepatitis B: Negative

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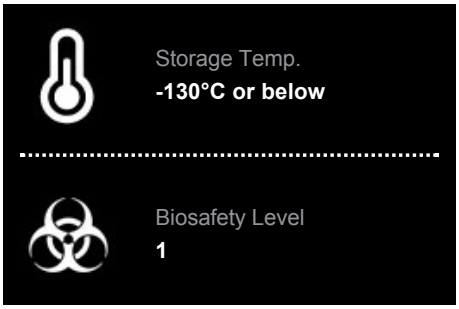

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Hepatitis C: Negative

Specific Staining
Hepatitis B: Negative
Hepatitis C: Negative
HIV: Negative

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 *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.

Human Material Precaution

All tissues used for isolation are obtained under informed consent and conform to HIPAA standards to protect the privacy of the donor's personal health information. It is best to use caution when handling any human cells. We recommend that all human cells be accorded the same level of biosafety consideration as cells known to carry HIV. With infectious virus assays or viral antigen assays, even a negative test result may leave open the possible existence of a latent viral genome.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. 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 strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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