





Product Sheet

Fibroblast Growth Kit-Serum-free (ATCC® PCS-201-040™)

Please read this FIRST



Storage Temp.
-20° (or -70°C for long-term storage)



Biosafety Level
1

Description

Product Description: Fibroblast Growth Kit–Serum-Free (ATCC® PCS-201-040) and Fibroblast Growth Kit–Low Serum (ATCC® PCS-201-041) each contain components that when added to Fibroblast Basal Medium (ATCC® PCS-201-030) create a complete ATCC® Primary Cell Solutions™ culture environment for fibroblasts (e.g., Dermal Fibroblasts, Normal, Human Neonatal [ATCC® PCS-201-010], Dermal Fibroblasts, Normal, Human Neonatal, Mitomycin C Treated [ATCC® PCS-201-010]).

Your experimental design will dictate which Fibroblast Growth Kit should be used. The use of Fibroblast Growth Kit–Serum-Free creates a completely defined medium for the serum-free culture of human fibroblasts. The rate of proliferation is equal to or greater than media supplemented using FBS (at concentrations ranging from 2% to 10%) through 10 population doublings. (Proliferation rate may slow under serum-free conditions after 10 population doublings.) Under these conditions, serum-free human feeder cell layers can be established for stem cell culture, or as a model system to study wound healing, skin aging, gene delivery, toxicology and basic questions of cell biology.

Use of the Fibroblast Growth Kit–Low Serum (ATCC® PCS-100-041) will support a faster rate of proliferation compared to Fibroblast Growth Kit–Serum-Free (ATCC® PCS-100-040). An upper limit for population doublings has not been established for primary fibroblasts when cultured with Fibroblast Basal Medium supplemented with Fibroblast Growth Kit–Low Serum (2% FBS). Under these conditions, primary fibroblast cultures have been maintained for 30+ population doublings, performing well when used as feeder layers to support human ES cell cultures.

Volume: 1 kit

Directions for Use

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. Store the basal medium at 2°C to 8°C and the growth kit(s) at either -20°C in a freezer that is not self-defrosting or at -70°C for long-term storage. If thawed upon arrival, the growth kit should be stored at 2°C to 8°C and added to the basal medium within 72 hours of receipt.

Preparation of Complete Growth Media

1. Obtain one growth kit from the freezer; make sure that the caps of all components are tight.
2. Thaw the components of the growth kit just prior to adding 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 Table 1 or 2, to the bottle of basal medium using a separate sterile pipette for each transfer.

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 basic | 0.5 mL | 5 ng/mL |
| rh EGF / TGF β-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 basic | 0.5 mL | 5 ng/mL |

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| | | |
|------------------------------|----------|----------|
| 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 |

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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.

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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.

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

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

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