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by life technologies

Keratinocyte-SFM (1X)

Description

Keratinocyte-SFM (serum-free medium) is optimized for the growth and maintenance of human keratinocytes and other types of epithelial cells without the need for a feeder layer of cells. Keratinocyte-SFM is a complete serum-free medium when supplemented with human recombinant Epidermal Growth Factor (rEGF) and Bovine Pituitary Extract (BPE) at the time of use. Both supplements, rEGF and BPE, are supplied in separate packaging with the basal medium. The complete medium may be used to cultivate human epidermal keratinocytes for studies involving dermal substitutes, *in vitro* toxicology, and gene therapy. This medium has been successfully used to cultivate cervical epithelial cells and is recommended for the culture of Human Corneal Epithelial Cells (HCECs). It may also be suitable as a medium for cultivating other epithelial cells, such as human bronchial epithelial cells, which have been shown to grow under serum-free conditions. Keratinocyte-SFM without calcium is available to allow researchers to adjust the Ca^{++} concentration to meet the demands of their specific application.

Product	Catalog no.	Amount	Storage	Shelf Life*
Keratinocyte-SFM (1X), liquid	17005-042	1 Kit		
Contains:				
Keratinocyte-SFM	10724-011	1 × 500 mL	2°C to 8°C; Protect from light	12 months
Keratinocytes Supplements	37000-015	1 Kit	–20°C to –5°C	18 months
Contains:				
Bovine Pituitary Extract (BPE)	13028-014	1 × 25 mg	–20°C to –5°C	18 months
EGF, Human Recombinant	10450-013	1 × 2.5 µg	–20°C to –5°C	18 months
Keratinocyte-SFM (1X), liquid w/o $CaCl_2$	37010-022	1 Kit		
Contains:				
Keratinocyte-SFM	10725-018	1 × 500 mL	2°C to 8°C; Protect from light	12 months
Keratinocytes Supplements	37000-015	1 Kit	–20°C to –5°C	18 months
Contains:				
Bovine Pituitary Extract (BPE)	13028-014	1 × 25 mg	–20°C to –5°C	18 months
EGF, Human Recombinant	10450-013	1 × 2.5 µg	–20°C to –5°C	18 months

* Shelf Life is determined from Date of Manufacture.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

Store the supplemented complete Keratinocyte-SFM Medium at 2°C to 8°C in the dark.

Safety Information

For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.

Supplement Media

Keratinocyte-SFM requires aseptic supplementation with rEGF and BPE before use.

1. Aseptically add BPE to Keratinocyte-SFM Basal Medium to a final concentration of 20-30 µg/mL. The concentration is listed on the BPE product label. **Note:** If preparing Keratinocyte-SFM for culture of HCECs, add the entire amount of BPE (25 mg) to 500 mL basal medium.
2. Aseptically add rEGF (0.1–0.2 ng/mL) to 500 mL Keratinocyte-SFM with BPE immediately before use in cell culture. **Note:** If preparing Keratinocyte-SFM for culture of HCECs, add the entire amount of rEGF (2.5 µg) to 500 mL basal medium.
3. Add antibiotics, if required. We recommend Gentamicin at 5 µg/mL.

Culture Conditions

Media: Complete Keratinocyte-SFM Medium

Cell Type: Human, epidermal keratinocytes, cervical epithelial cells, bronchial epithelial cells

Culture Type: Adherent

Recommended Culture Vessels: T-Flasks

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 4–6% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Cell Culture of Primary Human Keratinocytes

Prepare Human Foreskin tissue

1. At circumcision, place foreskins into complete Keratinocyte-SFM containing 5 µg/mL Gentamicin. Store foreskin tissue at 2°C to 8°C until use.

   Note: Human foreskins can be stored in complete Keratinocyte-SFM containing 5 µg/mL Gentamicin at 2°C to 8°C for approximately 5 days without significant loss of viable cell recovery.
2. Rinse foreskins with DPBS (Dulbecco's Phosphate Buffered Saline) without calcium and magnesium containing 20 µg/mL Gentamicin for approximately 1 hour.
3. Dissect foreskins into 2–4 pieces and transfer into a sterile 15-mL centrifuge tube.

Isolate Epidermal Keratinocytes

1. Prepare Dispase solution (25 caseinolytic units per mL dispase, 5 µg/mL Gentamicin in DPBS).

2. Submerge foreskin sections in Dispase solution and incubate for 18 hours at 2°C to 8°C.
3. Separate epidermal layer of human keratinocytes from the dermis and place into a sterile 15-mL conical tube containing 2 mL 0.05% Trypsin-EDTA.
4. Incubate at 36°C to 38°C for approximately 15 minutes. Triturate using a 2-mL pipette every 2–3 minutes to aid in cell dissociation.
5. Add 10 mL sterile filtered Soybean Trypsin Inhibitor (10 mg/mL in DPBS without calcium and magnesium).
6. Pellet cells by centrifuging at 180 × *g* for 7 minutes at room temperature.

Culture Primary Keratinocytes

1. Wash the cell pellet in 5–10 mL complete Keratinocyte-SFM medium and centrifuge 180 × *g* for 7 minutes at room temperature.
2. Resuspend the cell pellet in 5 mL complete Keratinocyte-SFM medium. Determine cell density using Countess® Automated Cell Counter.
3. Seed primary keratinocytes into T-75 culture flasks at a density of approximately 3×10^6 cells per flask in 15 mL complete Keratinocyte-SFM medium.
4. Exchange spent media with fresh complete Keratinocyte-SFM medium every 2–3 days.

   Note: Primary cultures may not reach 60–75% confluence until 10–20 days following isolation.

Secondary Culture of Human Epidermal Keratinocytes

Subculture keratinocytes directly into complete Keratinocyte-SFM medium. Ensure that cell confluency is between 60–75%, cell viability is at least 90%, and cell growth rate is in mid-logarithmic phase prior to secondary culturing.

1. Aspirate culture medium from cell monolayer and rinse with 10 mL DPBS without calcium and magnesium, aspirate and discard.
2. Add 1–2 mL 0.05% Trypsin-EDTA and incubate at 36°C to 38°C for 5–10 minutes. Observe the cell monolayer using an inverted microscope. When cells have rounded, aspirate Trypsin solution and reincubate until 90% of the cells have detached from the surface of the flask.
3. Add 10 mL sterile filtered Soybean Trypsin Inhibitor (10 mg/mL in DPBS without calcium and magnesium).
4. Transfer cell suspension into a sterile 15-mL centrifuge tube and centrifuge 180 × *g* for 7 minutes at room temperature.
5. Wash the cell pellet in 5–10 mL complete Keratinocyte-SFM medium and centrifuge 180 × *g* for 7 minutes at room temperature.
6. Resuspend the cell pellet in 5 mL complete Keratinocyte-SFM medium. Determine cell density using Countess® Automated Cell Counter.
7. Seed keratinocytes into T-75 culture flasks at a density of approximately $1–3 \times 10^6$ cells per flask in 15 mL complete Keratinocyte-SFM medium.
8. Exchange spent media with fresh complete Keratinocyte-SFM medium every 2–3 days until the cells reach 60–75% confluence, after which time cells can be further subcultured.

Cryopreservation

1. Obtain the appropriate volume of Synth-a-Freeze® cryopreservation medium and store at 2°C to 8°C until use.
2. Prepare the desired quantity of cells, harvest (steps 1–4 Secondary Culture of Human Epidermal Keratinocytes) in mid-log phase of growth with viability >90%. Determine cell density using Countess® Automated Cell Counter prior to centrifugation. **Note:** Typical cell densities for cryopreservation with Synth-a-Freeze® medium are 5×10^5 to 3×10^6 viable cells/mL.
3. Resuspend cell pellet in the pre-determined volume of 2°C to 8°C of Synth-a-Freeze® medium.
4. Immediately dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1 mL in a 2-mL cryovial).
5. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
6. Transfer frozen cells to liquid nitrogen. We recommend (vapor phase) storage at –200°C to –125°C.

Related Products

Product	Catalog No.
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190
Gentamicin Reagent Solution (50 mg/mL), liquid	15750
Dispase	17105
HBSS, calcium, magnesium, no phenol red	14025
TrypLE™ Express (1X), liquid, without Phenol Red	12563
Trypsin-EDTA, 1X	25300
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227
Human Corneal Epithelial Cells (HCEC)	C-018-5C
Synth-a-Freeze®, Defined Protein-Free Cryopreservation Medium	A12542

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

Caution, consult accompanying documents	Temperature Limitation	Sterilized using aseptic processing techniques	Consult instructions for use

Keep away from light	Catalog number	Manufacturer	Batch Code	Use By:

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.lifetechnologies.com/support**
For further assistance, email **techsupport@lifetech.com**