gibco by life technologies™

Defined Keratinocyte-SFM (1X), liquid

Description

Defined Keratinocyte- serum free medium (SFM) is optimized to support the growth and expansion of primary and secondary human keratinocytes without the use of fibroblast feeder layers. Defined Keratinocyte-SFM is a complete serum free medium consisting of Defined Keratinocyte Serum Free Basal Medium with the addition of Defined Keratinocyte-SFM Growth Supplement that eliminates the requirement for Bovine Pituitary Extract. The complete medium is recommended for culture of Human Corneal Epithelial Cells (HCECs) when used in combination with Coating Matrix Kit.

Product	Catalog no.	Amount	Storage	Shelf life*
Defined Keratinocyte-SFM	10744-019	1 kit	—	—
Kit Contains:				
Defined Keratinocyte SFM Basal Medium	10785-012**	1 × 500 mL	2°C to 8°C; Protect from light	12 months
Defined Keratinocyte-SFM Growth Supplement	10784-015**	1 × 1 mL	–20°C to –5°C; Protect from light	12 months

* Shelf life duration is determined from Date of Manufacture.

** Defined Keratinocyte-SFM Growth Supplement and Basal Medium are not sold separately.

Product use

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

Important information

Do Not Use This Product If:

- Packaging appears compromised.
- Defined Keratinocyte-SFM Growth Supplement is received thawed.
- Defined Keratinocyte-SFM Basal Medium appears cloudy or a visible precipitate is observed.

Use of Coating Matrix Kit may significantly enhance cell growth and attachment.

Safety information

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 HBsAg. Handle in accordance with established bio-safety practices.

Prepare media

Defined Keratinocyte-SFM requires supplementation with Defined Keratinocyte-SFM Growth Supplement.

Note: Avoid repeated freeze/thaw cycles of Defined Keratinocyte-SFM Growth Supplement and use immediately once thawed.

1. Thaw frozen Defined Keratinocyte-SFM Growth Supplement in a 37°C water bath. Mix by gentle pipetting.
2. Aseptically add 1 mL of Defined Keratinocyte-SFM Growth Supplement to 500 mL of Defined Keratinocyte-SFM Basal Medium before use. Rinse Defined Keratinocyte-SFM Growth Supplement vial with Basal Medium to ensure entire contents are transferred. Gently swirl to mix.
3. Add antibiotics, if required. It is recommended to use Gentamicin at 5 µg/mL.

Note: Once supplemented with Defined Keratinocyte-SFM Growth Supplement, the Complete Defined Keratinocyte-SFM is stable for 90 days when stored in the dark at 2°C to 8°C.

Culture conditions

Media: Complete Defined Keratinocyte-SFM Medium

Culture type: Adherent

Culture vessels: T-Flasks. Procedures described are for T-75 cm^2 flask cultures. Adjust volumes accordingly to culture vessel size.

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.

Culture of primary human keratinocytes

Prepare human foreskin tissue

1. At circumcision, place foreskins into complete Defined 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 Defined 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. **Note**: TrypLE™ Express (1X) may be substituted for Trypsin/EDTA.
4. Incubate at 36°C to 38°C for approximately 15 minutes. Aspirate 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). **Note:** substitute 10 mL DPBS without calcium or magnesium without Soybean Trypsin Inhibitor if using TrypLE™ Express.

6. Pellet cells by centrifuging at 180 × *g* for 7 minutes at room temperature.

Culture primary keratinocytes

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

Note: Primary cultures may not reach 60% to 75% confluence until 10–20 days following isolation. Use of Coating Matrix Kit to coat culture vessels with sterile recombinant human Type 1 collagen may significantly enhance cell attachment and growth.

Secondary culture of human epidermal keratinocytes

Subculture keratinocytes directly into complete Defined Keratinocyte-SFM medium. Ensure that cell confluency is between 60–75%, cell viability exceeds 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 37°C for 5–10 minutes. Observe 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. **Note**: TrypLE™ Express (1X) may be substituted for Trypsin/EDTA.
3. Add 10mL sterile filtered Soybean Trypsin Inhibitor (10 mg/mL in DPBS without calcium and magnesium). **Note:** substitute 10 mL DPBS without calcium or magnesium without Soybean Trypsin Inhibitor if using TrypLE™ Express.
4. Transfer cell suspension into a sterile 15-mL centrifuge tube and centrifuge at 180 × *g* for 7 minutes at room temperature.
5. Wash cell pellet in 5–10 mL complete Defined Keratinocyte-SFM medium and recentrifuge at 180 × *g* for 7 minutes at room temperature.
6. Resuspend cell pellet in 5 mL of complete Defined 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 of complete Defined Keratinocyte-SFM medium.
8. Exchange spent media with fresh complete Defined Keratinocyte-SFM medium every 2–3 days until the cells reach 60–75% confluence, after which time cells can be further subcultured.

Note: Use of Coating Matrix Kit to coat culture vessels with sterile recombinant human Type 1 collagen may significantly enhance cell attachment and growth.

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 1–5 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 $0.5–3 \times 10^6$ 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.
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.
DPBS, without calcium and magnesium	14190
Trypsin-EDTA, 1X	25300
Trypsin Inhibitor, Soybean	17075
TrypLE™ Express (1X), liquid, without Phenol Red	12604
Versene 1:5000, 1X	15040
Dispase	17105
Gentamicin	15750
Synth-a-Freeze®, Defined Protein-Free Cryopreservation Medium	A12542
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227
Coating Matrix Kit	R-011-K
Human Corneal Epithelial Cells (HCEC)	C-018-5C
Human Epidermal Keratinocytes, neonatal (HEKn)	C-001-5C

Explanation of symbols and warnings

The symbols present on the product label are explained below:

REF		MM/YYYY		LOT
Catalog number	Manufacturer	Use by	Protect from light	Batch code

	i		STERILE A
Caution, consult accompanying documents	Consult instructions for use	Temperature limitation	Sterilized using aseptic processing techniques

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**