GIBCO™
Invitrogen Corporation

Defined Keratinocyte-SFM

Serum-Free Keratinocyte Medium for the Culture of Human Keratinocytes

CAUTION: **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.**

Cat. No. 10744
Includes:

Cat. No.	**10785**	**Defined Keratinocyte-SFM**	**500 mL**
	10784	**Defined Keratinocyte-SFM Growth Supplement**	**1 mL**

Intended Use:

Defined Keratinocyte-SFM is used to support the growth of primary and secondary human keratinocytes. It is intended for laboratory research use only.

Background

Many systems have been described for the culture of human keratinocytes. Early work required serum-supplementation to media such as Medium 199[1] and NCTC 168[2]. Rheinwald and Green improved keratinocyte growth and colony formation by plating cells on lethally irradiated 3T3 fibroblasts and adding Epidermal Growth Factor (EGF) and hydrocortisone to the medium[3] . One of the first serum-free formulations developed was based on Medium 199 containing a growth factor cocktail which included bovine brain extract[4] . Serum-free cultivation of human keratinocytes without 3T3 fibroblast feeder layers became widely used with the development of MCDB-153[5] which included trace elements, ethanolamine, phosphoethanolamine, hydrocortisone, EGF and bovine pituitary extract (BPE). This medium has been subject to several enhancements[6,7,8] .
Serum-free culture in medium containing BPE as the primary mitogen has several drawbacks. The undefined composition of BPE complicates experimental models and interpretation of results. It may have either stimulatory or inhibitory effects on human keratinocyte cultures depending upon concentration and the presence of other components and their respective concentrations[9] and requires titration by the user into different systems. In addition, the stability of BPE in medium is limited to about four weeks.

Product Description

Defined Keratinocyte-SFM eliminates the requirement for BPE by inclusion of defined growth promoting additives including insulin, EGF and Fibroblast Growth Factor (FGF). The medium may be used for the establishment of primary keratinocytes as well as expansion of keratinocyte cultures. Defined Keratinocyte-SFM demonstrates superior primary cell growth while maintaining morphology and physiological markers.
Defined Keratinocyte-SFM is supplied as a kit containing 500mL liquid basal medium and a 1mL frozen growth supplement to be combined at the time of use.

Precautions

Aseptic addition of the Defined Keratinocyte-SFM Growth Supplement to Defined Keratinocyte-SFM basal medium is required before use. The growth supplement may have a clear to amber coloration, do not use if a precipitate is present.

Do Not Use This Product If:

1. Product packaging appears compromised.
2. Defined Keratinocyte-SFM Growth Supplement is received thawed.
3. Defined Keratinocyte-SFM basal medium appears cloudy or a visible precipitate is observed.

Storage

1. All products should be stored in the dark.
2. Store Defined Keratinocyte-SFM basal medium at 2 to 8°C.
3. Store Defined Keratinocyte-SFM Growth Supplement at -5 to -20°C.
4. Do not subject growth supplement to repeat freeze/thaw cycles.
5. Store the complete medium at 2 to 8°C in the dark.

Shelf-life

The unopened medium and growth supplement have a shelf-life of 12 months if stored in the dark at 2 to 8°C and -5 to -20°C, respectively. When stored properly, the complete medium has a shelf-life of at least 90 days under normal use conditions.

Instructions for Use

When ready to use, thaw the frozen supplement in a 37°C water bath and mix by gentle pipetting of the solution. Aseptically transfer the entire contents of the vial to the bottle of Defined Keratinocyte-SFM basal medium, rinse the vial with medium and add to the bottle of defined medium. Gently swirl the bottle of medium to ensure complete mixing.

Quality Control Testing

The growth-supporting ability of Defined Keratinocyte-SFM is tested using primary human keratinocytes isolated from neonatal foreskins and is compared against an in-house, previously approved, reference lot of Defined Keratinocyte-SFM. Briefly, human keratinocytes are plated into 6-well tissue culture plates at an initial seeding density of 1×10^4 cells per cm^2. Plates are incubated at 36°C ± 2°C in an atmosphere of 5% CO_2 in air for 2-3 days at which point they are fluid changed with fresh medium. Following a total of 6 days growth, cells are trypsinized and counted using a Coulter Model ZM Counter.

Cell Culture Methods for Human Keratinocytes

A. Isolation of epidermal keratinocytes from neonatal foreskins.
1. At circumcision, foreskins are placed into Defined Keratinocyte-SFM containing Gentamicin (Cat. No. 15710) at a concentration of 5 µg/mL. Foreskin tissue is then stored at 2 to 8°C until use. (Note: human foreskins can be stored in this holding medium at 2 to 8°C for approximately five days without significant loss of viable cell recovery.)
2. Foreskins are placed into a rinse solution of DPBS (without Ca^{++} or Mg^{++}) (Cat. No. 14190) containing Gentamicin at a concentration of 20 µg/mL for approximately 1 hour. Foreskins are cut into 2 to 4 pieces and transferred into a 25.0 caseinolytic units per mL solution of dispase (Cat. No. 17105) dissolved in DPBS and supplemented with Gentamicin at 5 µg/mL and incubated for 18 hours at 2 to 8°C.
3. After incubation in dispase, the epidermal layer of human keratinocytes is separated from the dermis and placed into a 60 mm petri dish containing 5-10 mL of 0.05% Trypsin-0.53 mM EDTA (Cat. No. 25300). The tissue is incubated at 36°C ± 2°C for approximately 15 minutes during which time it is aspirated using a 2mL pipette every 2-3 minutes to aid in cell dissociation.
4. Following incubation, trypsin activity is stopped by adding 10mL of Soybean Trypsin Inhibitor (Cat. No. 17075), at a final concentration of 10 mg/mL dissolved in D-PBS (without Ca^{++} and Mg^{++}) and sterile filtered prior to use. The cell suspension is transferred to a sterile 15 mL centrifuge tube and centrifuged at 40 x *g* for 5 minutes at room temperature. The resulting cell pellet is resuspended in medium and centrifuged as described above.
5. The keratinocyte pellet is gently resuspended in 5 mL of complete medium and the concentration of basal keratinocytes determined using a hemocytometer. Primary cells are seeded into 75 cm^2 tissue culture flasks at a density of approximately 3×10^6 cells per flask in 15 mL of complete medium. Cultures are incubated with a loosened cap at 36°C ± 2°C in a humidified atmosphere containing 5% CO_2 in air.
6. Keratinocyte cultures are fluid changed with fresh complete medium every 2-3 days.
7. Since primary keratinocyte cultures demonstrated donor to donor variability in their growth characteristics, primary cultures may not reach 60% to 75% confluence until 6 to 10 days following isolation and setup.

B. Secondary Culture of Human Epidermal Keratinocytes
1. Upon reaching 60% to 75% confluence, the culture medium is removed and the cell monolayer rinsed with 10 mL of Versene 1:5000 (Cat. No. 15040). One to 2 mL of 0.05% Trypsin- 0.53 mM EDTA is then added and the flask incubated for 5-10 minutes at 36°C ± 2°C. When cells have rounded, the trypsin is removed, and the flask reincubated at 36°C ± 2°C.
2. When approximately 90% of the cells have detached, the trypsin activity is stopped by the addition of 10 mL of Soybean Trypsin Inhibitor. The cell suspension is transferred to a sterile 15mL centrifuge tube and centrifuged at 40 x *g* for 5 minutes at room temperature. The cell pellet is gently resuspended in 5-10 mL of complete medium and recentrifuged at 40 x *g*.
3. The keratinocyte pellet is then resuspended in 3-5 mL of complete medium and the cells transferred into a 75cm^2 tissue culture flasks at a density of approximately 1-3 $\times 10^6$ cells per flask in 15 mL of complete medium.
4. Cultures are incubated in an atmosphere of 5% CO_2 in air at 36°C ± 2°C. Keratinocyte cultures are fluid changed every 2-3 days until the cells reach 60% to 75% confluence after which time cells can be further subcultured.

References

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