

DermaCult™ Keratinocyte Expansion Medium



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Catalog #100-0500

500 mL

Product Description

DermaCult™ Keratinocyte Expansion Medium is a serum- and BPE-free cell culture medium for feeder-free expansion of primary human keratinocytes. It supports > 50 population doublings of human neonatal keratinocytes and > 35 population doublings of human adult keratinocytes, while maintaining differentiation potential of the cells, such as the ability to form a stratified epithelium. Cells expanded in this medium exhibit cobblestone morphology and express key basal cell markers (K14, p63, ITAG6, and ITGB1) representative of basal keratinocytes, and are suitable for differentiation to air-liquid interface (ALI) cultures.

Product Information

The following components are sold as a complete kit (Catalog #100-0500) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
DermaCult™ Keratinocyte Expansion Basal Medium	100-0501	490 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
DermaCult™ Keratinocyte Expansion Supplement (50X)*	100-0502	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca ⁺⁺ and Mg ⁺⁺)	37350
Hydrocortisone Stock Solution	07925
ACCUASE™	07920
Trypan Blue	07050
Conical tubes, 15 mL	e.g. Catalog #38009

Preparation of Complete DermaCult™ Keratinocyte Expansion Medium

Use sterile technique to prepare complete DermaCult™ Keratinocyte Expansion Medium (Basal Medium + Expansion Supplement + Hydrocortisone Stock Solution). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw DermaCult™ Keratinocyte Expansion Supplement at room temperature (15 - 25°C). Mix gently by inverting the vial; do not vortex.
NOTE: Precipitate may be observed after thawing and can be resuspended by gentle mixing; this will not affect performance. Once thawed, use supplement immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 10 mL of DermaCult™ Keratinocyte Expansion Supplement and 0.5 mL of Hydrocortisone Stock Solution to 490 mL DermaCult™ Keratinocyte Expansion Basal Medium. Mix thoroughly.
NOTE: If not used immediately, store complete DermaCult™ Keratinocyte Expansion Medium at 2 - 8°C for up to 2 months. Do not exceed the shelf life of the individual components.
3. Complete medium does not contain antibiotics. If desired, add antibiotics immediately before use.

Directions for Use

Please read the entire protocol before proceeding. The following protocol is for expanding cultured primary human keratinocytes in a single well of a 6-well plate. If using other cultureware, adjust volumes and cell numbers accordingly.

NOTE: Only use tissue culture-treated cultureware. If using cells freshly isolated from tissue, additional optimization related to coating and plating density may be required.

1. Add $1 - 4 \times 10^4$ cells ($1.25 - 5 \times 10^3$ cells/cm²) in 2 mL of complete DermaCult™ Keratinocyte Expansion Medium to one well of a tissue culture-treated 6-well plate (e.g. Catalog #38015). Incubate at 37°C.

NOTE: If starting with cryopreserved keratinocytes, thaw cells directly into complete DermaCult™ Keratinocyte Expansion Medium and adjust seeding density according to post-thaw viability.

2. Perform a full-medium change every 2 days until cells are approximately 50 - 60% confluent and ready to be passaged; this typically takes 3 - 4 days.

NOTE: The expansion phase may take longer for some donor cell populations. Avoid allowing the culture to reach > 80% confluency, as it induces spontaneous differentiation of the cells.

3. Passage cells as follows:
 - a. Warm sufficient volumes of D-PBS (Without Ca⁺⁺ and Mg⁺⁺), complete DermaCult™ Keratinocyte Expansion Medium, and ACCUTASE™ to room temperature (15 - 25°C).
 - b. Wash cells with 2 mL of D-PBS (Without Ca⁺⁺ and Mg⁺⁺).
 - c. Add 0.5 mL of ACCUTASE™ to the well. Incubate at 37°C for 5 - 7 minutes, until cells can be dislodged with gentle tapping of the plate.
 - d. Add 2 mL of complete DermaCult™ Keratinocyte Expansion Medium to the well. Gently pipette up and down 3 - 5 times to achieve a single-cell suspension, then transfer cells to a 15 mL conical tube.
 - e. Centrifuge the tube at 180 x *g* for 7 minutes.
 - f. Remove and discard the supernatant. Resuspend the pellet in 1 mL of complete DermaCult™ Keratinocyte Expansion Medium.
4. Perform a viable cell count using Trypan Blue and a hemocytometer.

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