DermaCult™ Keratinocyte Expansion Medium

Serum- and BPE-free medium for expansion of primary human keratinocytes

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.

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>COMPONENT #</th>
<th>SIZE</th>
<th>STORAGE</th>
<th>SHELF LIFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DermaCult™ Keratinocyte Expansion Basal Medium</td>
<td>100-0501</td>
<td>490 mL</td>
<td>Store at 2 - 8°C.</td>
<td>Stable for 18 months from date of manufacture (MFG) on label.</td>
</tr>
<tr>
<td>DermaCult™ Keratinocyte Expansion Supplement (50X)*</td>
<td>100-0502</td>
<td>10 mL</td>
<td>Store at -20°C.</td>
<td>Stable for 18 months from date of manufacture (MFG) on label.</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-PBS (Without Ca++ and Mg++)</td>
<td>37350</td>
</tr>
<tr>
<td>Hydrocortisone Stock Solution</td>
<td>07925</td>
</tr>
<tr>
<td>ACCUTASE™</td>
<td>07920</td>
</tr>
<tr>
<td>Trypan Blue</td>
<td>07050</td>
</tr>
<tr>
<td>Conical tubes, 15 mL</td>
<td>e.g. Catalog #38009</td>
</tr>
</tbody>
</table>

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: A precipitate may be observed after thawing; 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 DermaCult™ Keratinocyte Expansion Supplement and 0.5 mL 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 x 10^4 cells (1.25 - 5 x 10^3 cells/cm²) in 2 mL 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 D-PBS (Without Ca++ and Mg++).
   - **c.** Add 0.5 mL 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 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 complete DermaCult™ Keratinocyte Expansion Medium.

4. **Perform** a viable cell count using Trypan Blue and a hemocytometer.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO WWW.STEMCELL.COM/COMPLIANCE.

Copyright © 2021 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, and DermaCult are trademarks of STEMCELL Technologies Canada Inc. ACCUTASE is a trademark of Innovative Cell Technologies, Inc. All other trademarks are the property of their respective holders. This product was developed under a license to intellectual property owned or controlled by Propagenix, Inc. This product is sold for research use only (which includes pre-clinical research) under a non-transferable, limited-use license. Purchase of this product does not include the right to sell, use or otherwise transfer this product for commercial purposes or clinical use. Purchasers wishing to use the product for purposes other than research use should contact Propagenix, Inc. (www.propagenix.com/about#contact-us). While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.