EPITHELIAL CELL CULTURE 2013/14

Precision Culture Media Tailored For Every Need:

• Retain proliferative progenitor cells
• Drive differentiation in 2D or 3D culture
• Age cells naturally without acute chemical treatments

Cells From a Variety of Epithelia:

• Primary human cells
• Long-term animal cells
• Suitable for 2D or 3D culture

Swiss Quality Products
Introduction

**CELLnTEC Story**

CELLnTEC was founded by a group of scientists from the University of Bern in 2002 in order to develop a new range culture media based on the most recent advances in our understanding of epithelial cell biology. Using our precision-media approach, we now provide a range of media tailored to the specific demands of either proliferative progenitor or fully differentiated epithelial cells.

CELLnTEC’s precision-media approach is based on a detailed understanding of the signaling pathways controlling progenitor cell maintenance and differentiation. Components of the cell’s micro-environment play a significant role in the regulation of these pathways.

R & D at CELLnTEC is dedicated to understanding in vivo cell environments in more detail, and transferring this knowledge directly into improved and specialized cell culture media. As a result, cells grown in our media provide precise and reliable results for your two dimensional (2D) in vitro experiments and accurate modeling of a in vivo-like situation in three dimensional (3D) cultures.

As a small, dynamic company, CELLnTEC continues to follow its original set of core values:

- Keep continually up to date with the latest science
- Provide top quality products together with tested protocols to maximize success
- Maintain an ongoing R & D program to develop new products, and refine existing ones
- Provide expert technical service through experienced scientists

**CELLnTEC Contacts**

We pride ourselves on the quality of our customer support. Please use the following email addresses to contact us:

Technical support and scientific questions: scientist@cellntec.com
Direct orders to CELLnTEC: orders@cellntec.com (see www.cellntec.com for countries served by distributors)
Contract research and testing: services@cellntec.com
General inquiries: info@cellntec.com
CELLnTEC - Precision Media

CELLnTEC’s precision media are designed to mimic specific micro environments in the body which play an important role in determining cell behavior. In this way our media are tailored to the particular requirements of either proliferative progenitors or fully differentiated cells. As a result CELLnTEC’s precision media deliver a host of benefits during the isolation, proliferation, and differentiation of epithelial cells.

PCT - Progenitor Cell Targeted

Progenitor Cell Targeted (PCT) media are designed to mimic the signaling environment of the stem cell niche more accurately than traditional media. As a result these media promote the establishment and retention of progenitor cells in vitro, and decrease their loss via differentiation in primary cell culture. Specific benefits include:

- **Improved Isolation:** by mimicking the in vivo progenitor cell micro-environment, PCT media establish more proliferative progenitor cells per isolation than traditional media (see Figure 1 for a comparison of different media).

- **Progenitor Cell Enrichment:** PCT media effectively retain progenitor cells in a proliferative state, resulting in more cells expressing desirable progenitor cell markers, without the need for a specific selection or cell sorting process (see Figure 2 for a FACS analysis of mouse keratinocytes).

- **Improved Longevity:** by mimicking the environment of the stem cell niche, PCT media are more effective at maintaining progenitors in a proliferative phenotype, resulting in fewer cells being lost to differentiation, and increased in vitro longevity of primary cell cultures.

Differentiation in 2D and 3D

Traditional cell culture media were developed over many years purely with the goal of improving cell growth. As a result, these media are generally not well suited to the specific demands of differentiating cells.

CELLnTEC’s non-PCT media are specifically designed to allow full differentiation. Characterization studies have confirmed the expression of late-stage differentiation markers in several epithelia in both 2D and 3D culture with CELLnTEC media.

Media used to establish stratified 3D cultures must create a very specific environment, that can maintain a population of proliferative cells in the basal layer, while at the same time allowing terminal differentiation in the upper layers of the model. CELLnTEC’s 3D Prime medium delivers exactly this balance. Read more on page 10.

Natural Aging In Vitro

Regular media have continuously evolved over five decades, and now provide rapid cell growth for extended periods. They contain many protective and stimulatory factors that have anti-aging effects. CELLnTEC’s new VitroAge medium is formulated without the high amounts of anti-aging ingredients found in standard media. In this medium, keratinocytes age naturally over several weeks, without the need for acute doses of pro-aging chemicals or oxidants. Read more on page 12.
**Cell Culture Media Overview**

**Media Selection Guide**

CELLnTEC’s precision media include PCT (Progenitor Cell Targeted) and non-PCT formulations. PCT Media provide improved isolation, proliferation and retention of proliferative progenitor cells. These media should be used for all isolation and routine primary cell culture work (available as completely defined and, in some cases, low-BPE containing versions). Non-PCT media are used for cultivation and allow late-stage differentiation of cells established in a PCT medium.

Overall CELLnTEC media may be divided into four main families:

1. **PCT Defined Media**: Specifically retain progenitor cells in a proliferative state. They offer high efficiency isolation and growth for primary epithelial cell culture in a completely defined environment.
2. **PCT Low-BPE Media**: Combine all the progenitor cell retention benefits of PCT with the growth and adhesion boost of BPE for the absolute best cell isolation and growth performance, or when the quality of the starting tissue is not optimal.
3. **Non-PCT Defined Media - 2D Differentiation**: Defined media used for cultivation and in situations where cells are induced to differentiate (high calcium trigger may be required, see protocols).
4. **Non-PCT Defined Media - 3D Differentiation**: Defined media used for differentiation of cells in three dimensional culture. Contains all the components to initiate differentiation, while keeping a subset of proliferation active cells. No further additions required.

<table>
<thead>
<tr>
<th>Tissue / Cells</th>
<th>Species</th>
<th>PCT Medium (Defined)</th>
<th>PCT Medium (Low BPE)</th>
<th>2D Differentiation (Defined)</th>
<th>3D Differentiation (Defined)</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratinocytes</td>
<td>Human / Mouse</td>
<td>CnT-07</td>
<td>CnT-57</td>
<td>CnT-02</td>
<td>CnT-02-3DP</td>
<td>7 / 10</td>
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<tr>
<td></td>
<td>Rat</td>
<td>CnT-03</td>
<td>CnT-33</td>
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<td>Keratinocytes (aged)</td>
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<td>12</td>
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<td>CnT-20</td>
<td>CnT-50</td>
<td>CnT-30</td>
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<td>CnT-32</td>
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<tr>
<td>Mammary</td>
<td>Human / Mouse</td>
<td>CnT-27</td>
<td>CnT-54</td>
<td>CnT-22</td>
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<td>Melanocyte</td>
<td>Human</td>
<td>CnT-12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CnT-52&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Prostate</td>
<td>Human / Mouse</td>
<td>CnT-11&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>CnT-17</td>
<td>CnT-23</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Airway</td>
<td>Human / Mouse</td>
<td>CnT-14</td>
<td>CnT-18</td>
<td>CnT-21</td>
<td></td>
<td>16</td>
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<tr>
<td>Bladder</td>
<td>Human / Mouse</td>
<td>CnT-16</td>
<td>CnT-58</td>
<td>CnT-36</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Vaginal</td>
<td>Human / Mouse</td>
<td>CnT-19</td>
<td>CnT-55</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Notes**: <sup>a</sup> Also used for differentiation experiments. <sup>b</sup>Contains 1% serum, no BPE

<table>
<thead>
<tr>
<th>Other Specialty Media (Non-PCT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue / Cells</td>
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<tr>
<td>Keratinocytes</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
</tr>
</tbody>
</table>

**Note**: <sup>i</sup> Contain serum. Used for isolation / proliferation

**Free Sample Program**

CELLnTEC provides a range of free samples to customers interested to test the performance of our new precision culture media in their own laboratory.

Catalog numbers and the contents of each sample pack can be found on the corresponding media pages of this catalog (see tables above for the corresponding media pages).

Important Note: When testing your free sample, there are a number of important considerations. Testing recommendations are provided with each sample pack, or can be found on CELLnTEC’s website.

www.cellntec.com
CELLnTEC Cell Overview

Primary Human Cells

CELLnTEC provides a range of primary human epithelial cells isolated directly in our precision PCT media.

Available from epidermis, cornea, bladder, and oral tissue, these cultures exhibit a high percentage of progenitor cells, excellent morphology, and extended longevity. Human dermal fibroblasts are also available.

All CELLnTEC primary cells are supplied as a kit with 500 mL of the appropriate culture medium. Kits are available in two sizes, with either > 0.5 million or > 1.5 million viable cells. For more details see the pages indicated below.

CELLnTEC primary cell systems come with our culture guarantee which states that successfully established cells, when following our protocols and using our media, will continue to proliferate for at least the number of additional population doublings indicated in the data sheet.

### Available Primary Human Cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal Keratinocytes (Single and Pooled Donors)</td>
<td>8</td>
</tr>
<tr>
<td>Dermal Fibroblasts (Single and Pooled Donors)</td>
<td>21</td>
</tr>
<tr>
<td>Corneal Epithelium Cells (Single Donor)</td>
<td>19</td>
</tr>
<tr>
<td>Gingival Epithelium Cells (Single and Pooled Donors)</td>
<td>27</td>
</tr>
<tr>
<td>Bladder Epithelium Cells (Single Donor)</td>
<td>17</td>
</tr>
</tbody>
</table>

**Note:** All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

Long-Term Animal Cells

CELLnTEC’s long-term cells are spontaneously immortalised cultures that provide the convenience of long-term cell growth without senescence. These cells have been isolated from normal tissue; they have not been actively transformed.

CELLnTEC’s animal cell systems express a range of cell markers, and can also be induced to differentiate. For example, extensive characterization of the MPEK mouse keratinocytes has shown signs of late stage differentiation up to passage 50 (see page 9 for a range tested markers).

Long-term cell systems are provided at approximately passage 25, and come with a guarantee of 6 months continuous growth after successful established, proliferating cultures following our protocols and using our media. In our tests these cells have grown much longer, and with appropriate frozen stocks, will be a reliable model for many years of research.

### Available Long-Term Animal Cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal Keratinocytes (Mouse, Rat, Dog)</td>
<td>9</td>
</tr>
<tr>
<td>Dermal Fibroblasts (Mouse, Rat)</td>
<td>21</td>
</tr>
<tr>
<td>Prostate Epithelium Cells (Rat)</td>
<td>29</td>
</tr>
<tr>
<td>Small Airway Epithelium Cells (Rat)</td>
<td>15</td>
</tr>
<tr>
<td>Bladder Epithelium Cells (Rat)</td>
<td>17</td>
</tr>
<tr>
<td>Vaginal Epithelium Cells (Rat)</td>
<td>31</td>
</tr>
</tbody>
</table>

**Note:** All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.
CELLnTEC provides a comprehensive range of specialized products for the culture of epidermal keratinocytes, including precision media, primary cells (see Figure 5), 3D culture products and a selection of general cell culture reagents (see page 32).

Our precision PCT media for the isolation of primary keratinocytes provide increased colony forming efficiency, homogeneous cobblestone morphology, progenitor cell enrichment, and extended longevity of human, mouse and rat keratinocytes.

Non-PCT Media are specifically designed to encourage complete differentiation in either 2D or 3D culture.

CELLnTEC also offers 3D starter kits that enable you to establish 3D epidermal keratinocyte cultures (see Figure 6) routinely in your own lab for a fraction of the price of purchased ready-to-use models. A full protocol and a complete training video can be found in the resources section of www.cellntec.com.

Natural keratinocyte aging is also now possible using the specialized VitroAge medium, in which keratinocytes age over several weeks, without the need for chemical stimuli or acute doses of oxidizers (see Page 12).

Don't Forget!
- Please visit the epidermal page on www.cellntec.com to obtain independent analyses comparing our media with the competition.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!

Figure 5: Human primary keratinocytes (HPEK) 5 days after thawing in CnT-07 (cells grown according to our recommended protocols and seeding densities)

Figure 6: 3D epidermal model (day 16) established using the 3D Starter Kit (see Page 11) and the complete protocol available on www.cellntec.com, where you will also find our 3D cell culture instructional video
Epidermal Keratinocyte Cell Culture

2D Culture Media

By specifically targeting the progenitor cells, PCT media enable optimal isolation and colony formation efficiency, and growth in either fully defined or low-BPE conditions, without the need for plate coating, feeder cells or other supplements. These formulations have a low calcium concentration that is necessary for cell growth without inducing differentiation.

In contrast, non-PCT media do not contain the components that specifically enhance the retention of progenitor cells. Using these media, cells can be induced to differentiate once confluent through the addition of calcium.

CELlnTEC’s specialized epidermal keratinocyte media are tailored to the specific needs of a variety of species, including human, mouse, rat, dog and sheep.

Isolation & Proliferation

**CnT-07; CnT-03** - Defined, PCT media. Where a completely defined environment and maximum keratinocyte purity is required. The PCT formulations of CnT-07 and CnT-03 provide excellent results through maximum retention of keratinocyte progenitors, and inhibition of fibroblasts.

**CnT-57** - Low-BPE, PCT medium recommended where absolute best attachment, isolation efficiency, colony formation efficiency and longevity is required, the quality of the starting material is not optimal, and / or a low BPE concentration (5 to 8 x lower than competitors) is compatible with the experimental setup.

**CnT-02; CnT-33** - Defined media using a optimized basal formulation and supplements. These media do not contain PCT factors, and are intended for use when keratinocytes are induced to differentiate.

Special Formulations

**CnT-AG2 Aging Medium** - Specifically designed to age keratinocytes naturally over several weeks without the need for acute doses of pro-aging chemicals or oxidizers. See page 12.

**CnT-02CF; CnT-07CF; CnT-57CF** - Calcium free formulations. Calcium must be added by the researcher for growth to occur in these media.

**Customized Formulations** - Available on request, e.g. phenol-red free, EGF free, hydrocortisone free etc.

**Important Note**

**Protocols:** Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELlnTEC website. Please send any technical questions direct to our team via scientist@cellntec.com.

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**Cat # | Description | Species | PCT | Defined**
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| CnT-57 | PCT Epidermal Keratinocyte Medium, Low BPE | Human / Mouse | ✓ | ✓ |
| CnT-57CF | PCT Epidermal Keratinocyte Medium, Low BPE, Ca Free | Human / Mouse | ✓ | ✓ |
| CnT-07 | PCT Epidermal Keratinocyte Medium, Defined | Human / Mouse | ✓ | ✓ |
| CnT-07CF | PCT Epidermal Keratinocyte Medium, Defined, Ca Free | Human / Mouse | ✓ | ✓ |
| CnT-02 | Epidermal Keratinocyte Medium, Defined | Human / Mouse | ✓ | ✓ |
| CnT-02CF | Epidermal Keratinocyte Medium, Defined, Ca Free | Human / Mouse | ✓ | ✓ |
| CnT-AG2 | PCT Epidermal Keratinocyte Aging Medium, Defined | Human | ✓ |
| CnT-03 | PCT Epidermal Keratinocyte Medium, Defined | Rat | ✓ |
| CnT-33 | Epidermal Keratinocyte Medium, Defined | Rat | ✓ |
| CnT-08 | Epidermal Keratinocyte Medium | Sheep | |
| CnT-09 | Epidermal Keratinocyte Medium | Dog | |

**Cat # | Description | Species | Media Included**
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| KMSPSAMPLE | Keratinocyte Media Sample Pack | Human / Mouse | CnT-07, CnT-57 |

**Note:** Samples include 100 mL of each medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antimycotic free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).
Epidermal Keratinocyte Cell Culture

Primary Human Keratinocytes

Isolated using our unique Progenitor Cell Targeted PCT media, CELLnTEC provides a range of primary human keratinocytes from single and pooled neonatal donors (see Figures 7 and 8), as well as cells from single adult donors.

The ability of PCT media to significantly increase colony formation efficiency during isolation ensures that retention of proliferative progenitor is maximized, and that the proliferative potential and longevity of these primary keratinocyte cultures is maintained at high levels.

All CELLnTEC primary cells are supplied as a kit, with a 500 mL bottle of the appropriate culture medium (basal medium with frozen supplements). In addition, most kits are available in two sizes, either 1 vial of > 5 x 10^5 viable cells, or alternatively 3 vials of > 5 x 10^5 viable cells. Cells are shipped under dry ice but must be stored e.g. liquid nitrogen upon arrival.

Furthermore, CELLnTEC primary keratinocyte progenitors come with our culture guarantee, which states that successfully established cells, following our protocols and using our media, will continue to proliferate for at least 20 additional population doublings.

CELLnTEC offers the following primary epidermal keratinocytes:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPEKp.05</td>
<td>Epidermal Keratinocyte Progenitors, Pooled (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-57</td>
</tr>
<tr>
<td>HPEKp.15</td>
<td>Epidermal Keratinocyte Progenitors, Pooled (3 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-57</td>
</tr>
<tr>
<td>HPEKs.05</td>
<td>Epidermal Keratinocyte Progenitors, Single Donor (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-57</td>
</tr>
<tr>
<td>HPEKs.15</td>
<td>Epidermal Keratinocyte Progenitors, Single Donor (3 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-57</td>
</tr>
<tr>
<td>HPEKas.05</td>
<td>Epidermal Keratinocyte Progenitors, Adult, Single Donor (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-57</td>
</tr>
<tr>
<td>HPEKas.15</td>
<td>Epidermal Keratinocyte Progenitors, Adult, Single Donor (3 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-57</td>
</tr>
</tbody>
</table>

Note: All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

Figure 7: Human Epidermal Keratinocytes (HPEKp) 4 days after thawing in CnT-57 (cells grown according to our recommended protocols and seeding densities)

Figure 8: Human Epidermal Keratinocytes (HPEKp) in CnT-57 after 20 population doublings (cells grown according to our recommended protocols and seeding densities)
Epidermal Keratinocyte Cell Culture

Long-Term Animal Cells

CELLnTEC's long-term keratinocytes (see Figures 9 and 10 for mouse cells) are spontaneously immortalized cells that provide the convenience of long-term cell growth without senescence. These cells have been isolated from normal tissue and have not been actively transformed.

Long-term keratinocyte cultures accurately model many biological processes, and have been widely used in investigations ranging from wound healing, to cell signaling and differentiation.

For additional information on these cells, please visit the epidermal products section of www.cellntec.com. Further information on the use of these products in the literature can also be found in our publications database.

All CELLnTEC long-term cell systems are supplied as a kit containing cryo preserved cells (approximately passage 25) and a bottle of the appropriate culture medium. Cells come with a long-term guarantee (see Page 5).

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
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</thead>
<tbody>
<tr>
<td>MPEK-BL6</td>
<td>Epidermal Keratinocyte Progenitors (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Mouse, C57 / BL6</td>
<td>CnT-07</td>
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<td>MPEK-129</td>
<td>Epidermal Keratinocyte Progenitors (1 x &gt; 5 x 10^5 viable cells)</td>
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<td>CnT-07</td>
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<td>RPEK</td>
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<td>CnT-03</td>
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<td>CPEK</td>
<td>Epidermal Keratinocyte Progenitors (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Dog, Beagle</td>
<td>CnT-09</td>
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</tbody>
</table>

Note: All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

General Cell Culture Reagents: See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE and stains.

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Figure 9: Mouse epidermal long-term keratinocytes (MPEK-BL6), 3 day after thawing in CnT-07 (cells grown according to our recommended protocols and seeding densities)

Figure 10: Mouse epidermal long-term keratinocytes (MPEK-BL6), 6 days after thawing in CnT-07 (cells grown according to our recommended protocols and seeding densities)

Markers expressed in C57BL/6 murine long-term keratinocytes MPEK-BL6 for at least 25 passages:

<table>
<thead>
<tr>
<th>Proliferation</th>
<th>Differentiation</th>
<th>Armadillo</th>
<th>Cadherins</th>
</tr>
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<tbody>
<tr>
<td>Keratin 14</td>
<td>Loricrin</td>
<td>β-Catenin</td>
<td>E-Cadherin</td>
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<td>β1-Integrin</td>
<td>Filaggrin</td>
<td>Plakoglobin</td>
<td>Desmoglein 1 / 3</td>
</tr>
<tr>
<td></td>
<td>Involucrin</td>
<td>Plakophilin 1 / 3</td>
<td>Desmocollin 1 / 2 / 3</td>
</tr>
<tr>
<td></td>
<td>Keratin 1</td>
<td>p120</td>
<td></td>
</tr>
</tbody>
</table>
Three dimensional (3D) epidermal keratinocyte cultures remain the gold-standard for highly accurate in vitro modeling of the epidermis (see Figure 11 for a lipid profile comparison). With a fully stratified structure extending from proliferative basal progenitors through fully differentiated cornified cells, these models are able to reproduce most of the intricate functions of the in vivo epidermis.

However the establishment of such a stratified model places very specific and quite divergent demands on the cell culture medium. In particular; the medium must support cells to reach terminal differentiation as they stratify and form the suprabasal layers of the model, whilst in parallel allowing a population of proliferative cells in the basal layer.

CELLnTEC is the first company to provide a specialized culture medium specifically tuned to the unique demands of a 3D keratinocyte culture. Known as 3D Prime, this fully defined medium was developed using our unique signaling based approach. It enables the routine and reliable establishment of a fully stratified epithelium in just 14 to 18 days air-lifted culture (see Figure 12).

The 3D Prime medium is also available as part of a kit containing all the components necessary for the establishment of 3D epidermal models in your own lab. See page 11 for more details.

Also available: a full training video demonstrating our recommended written protocol for the establishment of 3D epidermal cell cultures. Please visit www.cellntec.com.

### Cat # | Description | Species | Pack Size
--- | --- | --- | ---
CnT-02-3DP1 | Epidermal Keratinocyte 3D Prime Medium, Defined | Human | 100 mL
CnT-02-3DP5 | Epidermal Keratinocyte 3D Prime Medium, Defined | Human | 500 mL

**Note:** All media provided as kits containing 100 or 500 mL basal medium and frozen supplements.

### Free Sample

To test the benefits of our 3D Prime Media in your own lab, CELLnTEC offers a free sample kit, consisting of everything needed to successfully build a 3D epidermal cell culture with your own cells. The kit consists of 100 mL completely supplemented CnT-57 for isolation and cultivation of primary keratinocytes, 100 mL completely supplemented CnT-02-3DP Epidermal Keratinocyte Prime Medium, 6 inserts for air-liquid-interface culture and 1 mL each of Stain Solution I and II for the confirmation of confluency. The protocol for the successful establishment of a 3D culture is very important and should be followed closely. Please visit the resources section on our website for a 3D instructional video and our written protocol.

### Cat # | Description | Species | Media Included
--- | --- | --- | ---
CnT-02-3DPSAMPLE | Epidermal 3D Prime Sample Kit | Human | CnT-57, CnT-02-3DP

**Note:** Samples include 100 mL of each medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antimycotic free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).
Epidermal Keratinocyte Cell Culture

3D Cell Culture Starter Kits

For your convenience, you can obtain everything you need to reliably establish 3D epidermal models routinely in your own lab, to your own schedule, in one kit.

Kits are available with or without primary HPEK keratinocytes, and in two pack sizes (25 or 50 inserts).

CELLnTEC’s 3D starter kits contain:

- PCT Epidermal Keratinocyte Medium CnT-57 for isolating and maintaining primary keratinocytes prior to 3D culture (1 x 500 mL)
- 3D Prime Medium for air-liquid interface cell culture (2 x CnT-02-3DP1 in the 25, or 1 x CnT-02-3DP5 in the 50 inserts kit)
- Primary Keratinocytes (HPEKp.05; optional, not required if early passage cells growing in a PCT medium are used instead)
- Inserts with a porous polycarbonate membrane for air-liquid-interface culture
- Stain Solution I and II for confirming confluency (3 mL each)

For a complete demonstration of our protocols, please see the 3D training video on www.cellntec.com. In the resources section of the CELLnTEC website, protocols are available for routine histology and staining. For additional information or detailed questions, please contact one of our scientists: scientist@cellntec.com

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>HPEK Primary Cells</th>
<th>Number of Inserts</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D-HPEK-25</td>
<td>3D Keratinocyte Starter Kit including Human Primary Keratinocytes</td>
<td>✓</td>
<td>25</td>
</tr>
<tr>
<td>3D-HPEK-50</td>
<td>3D Keratinocyte Starter Kit including Human Primary Keratinocytes</td>
<td>✓</td>
<td>50</td>
</tr>
<tr>
<td>3D-K-25</td>
<td>3D Keratinocyte Starter Kit without Human Primary Keratinocytes</td>
<td>✓</td>
<td>25</td>
</tr>
<tr>
<td>3D-K-50</td>
<td>3D Keratinocyte Starter Kit without Human Primary Keratinocytes</td>
<td>✓</td>
<td>50</td>
</tr>
</tbody>
</table>

Note: Kits consist of the components indicated in the bullet list above this table.

General Cell Culture Reagents: See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.

Figure 13: Immunohistological stainings of in vitro epidermal models created with CELLnTEC’s 3D Keratinocyte Starter Kit. The 3D model establishes all the strata of the epidermis (stratum basale = SB, stratum spinosum, stratum granulosum = SG, stratum corneum = SC), and expresses a range of proliferation and differentiation markers (Ki67 and Keratin-10 shown above)
**Epidermal Keratinocyte Cell Culture**

**Natural Aging with VitroAge**

Standard culture media have evolved over 5 decades to deliver rapid in vitro cell growth over extended periods. They contain high doses of protective and stimulatory ingredients that have strong anti-aging effects.

As a result of the design of standard media, traditional methods to age keratinocytes in vitro have depended on the use of acute doses of pro-aging chemicals or strong oxidisers to induce some form of stress-induced aging. However, these approaches are compromised by the non-physiological nature of the pro-aging treatments, and the strong resistance that standard culture media have to the aging process.

The new VitroAge medium (CnT-AG2) developed by CELLnTEC is specifically formulated with respect to keratinocyte aging. It does not contain the high concentrations of protective and stimulatory factors found in standard media.

Keratinocytes growing in VitroAge medium age naturally over several weeks, without the need for acute or artificial chemical treatments. Cells retain normal morphology, but demonstrate key aging features seen in vivo, including fewer and slower cell divisions [1] and disrupted metabolism [2]. They also show a group of changes at the protein level (see page 13).

Following transfer from standard CnT-07 medium to the VitroAge medium (CnT-AG2), the proliferation rate of the keratinocytes decreases step-wise at each passage, until only minimal growth is observed after 3-4 passages.

---

**Control:** Keratinocytes growing in (standard) CnT-07 medium reach 100% confluency 6 days post seeding (passage 3).

**Aged:** Keratinocytes demonstrate slow growth during the 3rd passage of culture in CnT-AG2 medium (6 days post seeding, approx 35% confluent).

---

1. Charruyer, A et al. Transit-amplifying cell frequency and cell cycle kinetics are altered in aged epidermis. JID 129, 2574-2583 (2009)
Epidermal Keratinocyte Cell Culture

**VitroAge and Proteomics**

After three weeks of growth in VitroAge medium, in addition to slowing proliferation keratinocytes also display a group of age-related changes in their proteome.

Using a novel MRM proteomics technology, up to 100 proteins can now be accurately characterized in parallel in a single sample. Using this approach, we have identified 6 groups of age-related proteins that change significantly during 3 weeks of culture in VitroAge (CnT-AG2) culture medium:

- DNA repair mechanisms
- Detoxification mechanisms
- Antiox / Redox mechanisms
- Stress responses and proteasome
- Stem / Progenitor cell markers
- Metabolism / Protein synthesis

By characterizing 5-15 proteins in each group, a more detailed insight into the effect of an active ingredient can be obtained.

**Aging Overview:** Age-related proteomic changes in keratinocytes grown in VitroAge medium (CnT-AG2), with or without active ingredient. Changes in protein expression are denoted in percent, vs control (keratinocytes grown in CnT-07 standard medium). Percentages are absolute values (up or down regulation).

**Specific Aging Insights:** Proteomic changes can be quantified for specific groups, or individual proteins. Percentage changes (up or down regulation) are shown relative to keratinocytes grown in CnT-07 standard medium.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-AG2</td>
<td>PCT Epidermal Keratinocyte Aging Medium, Defined</td>
<td>Human</td>
<td>✅</td>
<td>✅</td>
</tr>
</tbody>
</table>

**Note:** Media provided as kits containing both 500 mL basal medium and frozen supplements.

**General Cell Culture Reagents:** See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.

**Free Samples**

CELLnTEC offers a free sample pack of the following media:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Media Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGSAMPLE</td>
<td>Epidermal Keratinocyte Aging Medium Sample Pack</td>
<td>Human</td>
<td>CnT-AG2</td>
</tr>
</tbody>
</table>

**Note:** AGSAMPLE includes 500 mL of CnT-AG2 medium. It comes completely supplemented, ready to use – no need for additional components. The medium is antibiotic / antimycotic free and has a shelf life of 6 weeks after production (stored at 4°C in the dark).
**Introduction**

The airway epithelium extends from the nose and mouth down to the alveoli, and features a muco-ciliary structure in its upper sections transitioning to a thin squamous epithelium in the alveoli.

CELLnTEC’s fully defined PCT media have been found to offer high isolation efficiency and growth of human, mouse and rat airway epithelial cells, without the need for non-defined additives such as BPE or serum. In addition, cells isolated in a PCT medium have also been shown to retain the ability to further differentiate in 3D cultures when grown at the air-liquid interface.

Direct comparisons have consistently found that defined PCT media deliver equal or better cell growth than several commonly used non-defined media. They can also be used for the cultivation of normal and diseased cell lines. For more information, please visit the airway section of www.cellntec.com.

**Don’t Forget!**

- Please visit the airway page on www.cellntec.com to obtain further information on CELLnTEC’s airway products.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!

![Figure 14: Human small airway epithelium cells in CnT-17, after 4 population doublings (cells grown according to our recommended protocols and seeding densities)](image1)

![Figure 15: Human small airway epithelium cells in CnT-17, after 5 population doublings (cells grown according to our recommended protocols and seeding densities)](image2)
Airway Epithelium Cell Culture

Culture Media

By specifically targeting the progenitor cells, PCT media enable optimal isolation, colony forming efficiency, and growth in a fully defined environment, without the need for plate coating, feeder cells or other supplements. These formulations have a low calcium concentration that is necessary for cell growth without inducing differentiation.

In contrast, non-PCT media do not contain the components that specifically enhance the retention of progenitor cells. Using these media, cells can be induced to differentiate once confluent through the addition of calcium.

CELLnTEC provides specialized airway epithelium media tailored to the specific needs of various species, including human, mouse, and rat.

Isolation & Proliferation

CnT-17; CnT-14 - Defined, PCT media which deliver maximum isolation efficiency and purity of human, mouse or rat airway epithelial cells due to the high retention of undifferentiated progenitor cells. CnT-17 offers excellent isolation and growth of both human large and small airway epithelial cells, while CnT-14 is optimized for rat small airway epithelium.

Differentiation

CnT-23; CnT-34 - Defined, non-PCT media. Intended for use in situations where cells are induced to differentiate, for example with calcium, for both 2D and 3D differentiation experiments.

Special Formulations

Customized Formulations - Available on request for most media; please contact us for more details. Some standard variations are routinely available, e.g. phenol-red free, EGF free, hydrocortisone free etc.

Important Note

Protocols: Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-17</td>
<td>PCT Airway Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CnT-23</td>
<td>Airway Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CnT-14</td>
<td>PCT Small Airway Epithelium Medium, Defined</td>
<td>Rat</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CnT-34</td>
<td>Small Airway Epithelium Medium, Defined</td>
<td>Rat</td>
<td></td>
<td>✔</td>
</tr>
</tbody>
</table>

Note: All media provided as kits containing both 500 mL basal medium and frozen supplements.

Free Samples

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMSPSAMPLE</td>
<td>Airway Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-17</td>
</tr>
</tbody>
</table>

Note: Samples include 100 mL of each medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antifungal free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).

Long-Term Animal Cells

CELLnTEC’s long-term cell systems are your choice where the convenience of long-term growth is required. These cells have been isolated from normal tissue, and have not been actively transformed.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSAK-WIS</td>
<td>Small Airway Epithelium Progenitors, Wistar (1 x &gt; 5 x 10⁶ viable cells)</td>
<td>Rat</td>
<td>CnT-14</td>
</tr>
</tbody>
</table>

Note: All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

General Cell Culture Reagents: See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.
Bladder Epithelium Cell Culture

Introduction

The epithelial lining of the bladder, known as the urothelium, is a transitional epithelium consisting of 3 to 5 cell layers. It is found in the bladder and sections of adjoining ducts such as the urethra and prostate. This specialized epithelium is highly elastic, and its function is to provide protection against the toxic components found in urine.

Progenitor Cell Targeted (PCT) media from CELLnTEC are designed to create a precise micro-environment that actively selects and encourages growth of proliferative urothelial progenitor cells. As a result, these media enable high isolation efficiency and early passage cell growth.

PCT media can be used successfully with human, mouse and rat tissue. In addition, cells isolated in a PCT medium also retain the ability to grow in 3D structures and form a stratified epithelium. For more complete information and insights from the literature, please visit www.cellntec.com.

In addition to specialized media, CELLnTEC also provides early passage human bladder epithelial cells (HBEP; see Figures 16 and 17), as well as long-term cells isolated from rat. More information can be found on the adjacent page.

Don’t Forget!

- Please visit the bladder epithelium page on www.cellntec.com to obtain further information on CELLnTEC’s bladder products.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!

Figure 16: Human bladder epithelium cells growing in the defined PCT medium CnT-18, 2 days after thawing (cells grown according to our recommended protocols and seeding densities)

Figure 17: Human bladder epithelium cells growing in clumps in the defined PCT medium CnT-18, 6 days after thawing (cells grown according to our recommended protocols and seeding densities)
Bladder Epithelium Cell Culture

Culture Media

CELLnTEC provides specialized bladder epithelium media tailored to the specific needs of human, mouse and rat bladder cells.

Isolation & Proliferation

CnT-18; CnT-16 - Defined, PCT media. Where a completely defined environment and maximum bladder epithelium cell purity is required. Both media provide excellent results through maximum retention of proliferative progenitors.

CnT-58 - Low-BPE, PCT medium recommended where absolute best attachment, isolation efficiency, colony formation efficiency and longevity is required, the quality of the starting material is not optimal, and / or a low BPE concentration (5 to 8 x lower than competitors) is compatible with the experimental setup.

Differentiation

CnT-21; CnT-36 - Defined media using highly optimized basal formulations and supplements, but without PCT factors. Intended where bladder cells are induced to differentiate.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-18</td>
<td>PCT Bladder Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CnT-58</td>
<td>PCT Bladder Epithelium Medium, Low BPE</td>
<td>Human / Mouse</td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>CnT-21</td>
<td>Bladder Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CnT-16</td>
<td>PCT Bladder Epithelium Medium, Defined</td>
<td>Rat</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>CnT-36</td>
<td>Bladder Epithelium Medium, Defined</td>
<td>Rat</td>
<td></td>
<td>✔</td>
</tr>
</tbody>
</table>

Note: All media provided as kits containing both 500 mL basal medium and frozen supplements.

Free Samples

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMSPSAMPLE</td>
<td>Bladder Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-58</td>
</tr>
</tbody>
</table>

Note: Samples include 100 mL of medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antimycotic free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).

Primary Human Cells

CELLnTEC has the following primary bladder epithelium cells available:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBEP.05</td>
<td>Bladder Epithelium Progenitors, Single Donor (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-58</td>
</tr>
<tr>
<td>HBEP.15</td>
<td>Bladder Epithelium Progenitors, Single Donor (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-58</td>
</tr>
</tbody>
</table>

Note: All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

Long-Term Animal Cells

CELLnTEC's long-term cell systems are your choice where the convenience of long-term growth is required. These cells have been isolated from normal tissue, and have not been actively transformed.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBVAK-WIS</td>
<td>Balder Epithelium Progenitors, Wistar (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Rat</td>
<td>CnT-16</td>
</tr>
</tbody>
</table>

Note: All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

Important Note

Protocols: Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended special bladder protocols found on the CELLnTEC website. For technical and scientific questions please contact...
Introduction

Proliferative corneal epithelium progenitors are found in the limbal region of the cornea. CELLnTEC’s Progenitor Cell Targeted (PCT) media create an environment specifically tailored to the needs of these proliferative progenitor cells.

Independent studies have shown that the PCT media CnT-20 and CnT-50 provide up to double the colony formation rate and in vitro longevity than traditional serum-containing media (see Figure 19 for an extract of the full poster available on www.cellntec.com). The cultures express high levels of stem and progenitor markers, in parallel with very low expression of differentiation markers.

CELLnTEC’s PCT media can be used to isolate primary corneal epithelial cells from both mouse and human tissue. In addition, these cells can also be induced to differentiate in either 2D or 3D culture.

Primary human corneal epithelial cells (HCEP; see Figure 18) are also available from CELLnTEC. Isolated in a PCT medium, these cells provide excellent morphology and longevity, and come with our culture guarantee.

CnT-20
Defined

CnT-50
Low-BPE

KSFM
BPE

SHEM
5% FBS

Don’t Forget!
- Please visit the corneal epithelium page on www.cellntec.com to obtain independent analyses comparing our media with the competition.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!
Corneal Epithelium Cell Culture

**Culture Media**

By targeting the progenitor cell population in a tissue sample, CELLnTEC’s Progenitor Cell Targeted (PCT) media offer significant improvements in isolation efficiency, growth and longevity over traditional formulations. For full comparison info, please visit www.cellntec.com.

**Isolation & Proliferation**

**CnT-20** - Defined, PCT medium. Where a completely defined environment and maximum corneal epithelium cell purity is required, the PCT formulation of CnT-20 provides excellent results through maximum retention of corneal epithelial progenitor cells.

**CnT-50** - Low BPE, PCT medium recommended where absolute best attachment and isolation efficiency, colony formation and longevity is required, and a low BPE concentration (5-8 x lower than competitors) is compatible with the experimental setup.

**Differentiation**

**CnT-30** - Defined medium using a highly optimized basal formulation and supplements, but does not contain PCT factors. Intended for use in situations where corneal cells are induced to differentiate, to avoid the differentiation delay which may occur with PCT media.

**Special Formulations**

**Customized Formulations** - Available on request for most media; please contact us for more details. Some standard variations are routinely available, e.g. phenol-red free, EGF free, hydrocortisone free etc.

**Important Note**

**Protocols:** Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-20</td>
<td>PCT Corneal Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CnT-50</td>
<td>PCT Corneal Epithelium Medium, Low BPE</td>
<td>Human / Mouse</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CnT-30</td>
<td>Corneal Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** All media provided as kits containing both 500 mL basal medium and frozen supplements.

**General Cell Culture Reagents:** See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.

**Free Samples**

CELLnTEC offers a free sample of the following media:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMSPSAMPLE</td>
<td>Corneal Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-20, CnT-50</td>
</tr>
</tbody>
</table>

**Note:** Samples include 100 mL of each medium and are completely supplemented. They are ready to use — no need for additional components. They are antibiotic / antymycotic free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).

**Primary Human Cells**

CELLnTEC’s primary human corneal keratinocytes are isolated in our specialized PCT culture medium CnT-20 or CnT-50, ensuring maximum retention of the valuable colony forming efficient progenitor cells and in vitro longevity.

Independent tests (Chuang et al. 2007) found primary corneal keratinocytes isolated in PCT media to offer better growth rate, colony forming efficiency and stem cell marker expression than a variety of competing media from various suppliers. For more details, and a searchable publications database, please visit www.cellntec.com.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCEP.05</td>
<td>Corneal Epithelium Progenitors, Single Donor (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-20 or CnT-50*</td>
</tr>
<tr>
<td>HCEP.15</td>
<td>Corneal Epithelium Progenitors, Single Donor (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-20 or CnT-50*</td>
</tr>
</tbody>
</table>

**Note:** All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

* Lot dependent. Please contact orders@cellntec.com for further information.
Fibroblast Cell Culture

Introduction

Fibroblasts are cells found in connective tissue throughout the body. In addition to their physical role in the maintenance of tissue structure, it is widely known that these cells also play a significant role in the behavior of adjacent epithelial cells via a complex network of intercellular signals.

Culture Media

CELLnTEC offers finely tuned media for both 2D and 3D fibroblast culture

For 2D culture, CnT-05 is recommended (Fig 20.1). This medium features an optimized basal medium supplemented with both serum and several proprietary growth factors.

As a result of these factors, CnT-05 enables excellent isolation and growth of fibroblasts from a wide range of tissues, including skin, airway, cornea, bladder and vagina, from multiple species including human, mouse, rat and rabbit.

For 3D spheroid culture the new fully defined CnT-DP-3D medium is recommended. This medium enables the establishment and passaging of spheres, resulting in significantly improved expression of progenitor cell markers such as Sox-2 (see Figure 20.2).

In addition to our culture media, CELLnTEC also provides primary human dermal fibroblasts or dermal papilla progenitors (see page 21), as well as long-term fibroblast cultures from mouse and rat tissue.

For fully detailed product information, protocols and the latest results from the literature, please visit www.cellntec.com.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-05</td>
<td>Fibroblast Medium</td>
<td>Human / Mouse / Rat / Rabbit</td>
<td></td>
</tr>
<tr>
<td>CnT-DP-3D</td>
<td>3D Fibroblast Medium</td>
<td>Human / Mouse</td>
<td>✓</td>
</tr>
</tbody>
</table>

Note: Medium provided as a kit containing both 500 mL basal medium and frozen supplements.

Free Samples

CELLnTEC offers a free sample of the following media:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMSPSAMPLE</td>
<td>Fibroblast 3D Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-DP-3D; CnT-05</td>
</tr>
</tbody>
</table>

Note: Samples include 100 mL of each medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antifungal free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).
Fibroblast Cell Culture

Primary Human Cells

CELLnTEC primary dermal fibroblasts (HDF) are isolated in our specialized culture medium CnT-05, ensuring maximum cell growth and in vitro longevity.

Dermal progenitor cells isolated from the dermal papilla (HDP) are also available.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDFs.05</td>
<td>Dermal Fibroblasts, Single Donor (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-05</td>
</tr>
<tr>
<td>HDFs.15</td>
<td>Dermal Fibroblasts, Single Donor (3 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-05</td>
</tr>
<tr>
<td>HDFp.05</td>
<td>Dermal Fibroblasts, Pooled (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-05</td>
</tr>
<tr>
<td>HDFp.15</td>
<td>Dermal Fibroblasts, Pooled (3 x &gt; 5 x 10^5 viable cells)</td>
<td>Human</td>
<td>CnT-05</td>
</tr>
<tr>
<td>HDPs.05</td>
<td>Dermal Progenitors, Single Donor</td>
<td>Human</td>
<td>CnT-05</td>
</tr>
</tbody>
</table>

**Note:** All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

Long Term Animal Cells

CELLnTEC’s long-term cell systems are your choice where the convenience of long-term growth is required. These cells have been isolated from normal tissue, and have not been actively transformed (see Figures 21 and 22 for mouse long-term fibroblasts).

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF-BALBC</td>
<td>Dermal Fibroblasts, BALBC (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Mouse</td>
<td>CnT-05</td>
</tr>
<tr>
<td>DF-R</td>
<td>Dermal Fibroblasts, Wistar (1 x &gt; 5 x 10^5 viable cells)</td>
<td>Rat</td>
<td>CnT-05</td>
</tr>
</tbody>
</table>

**Note:** All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

General Cell Culture Reagents: See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.

Figure 21: Long-term murine dermal fibroblasts (DF-BALBC) one day after thawing in CnT-05 (cells grown according to our recommended protocols and seeding densities)

Figure 22: Long-term murine dermal fibroblasts (DF-BALBC) 5 days after thawing in CnT-05 (cells grown according to our recommended protocols and seeding densities)
Introduction

By targeting the specialized needs of the mammary epithelium progenitor cells, CELLnTEC’s Progenitor Cell Targeted (PCT) media are able to improve isolation efficiency and morphology, and offer extended longevity.

PCT media for mammary epithelium are available in both fully defined and low-BPE formulations. In addition, an alternative non-PCT formulation is also available in which cells can be induced to differentiate. PCT media can be used to grow cells as monolayers, or alternatively as spheres. For further details, please visit www.cellntec.com.

Don’t Forget!

- Please visit the mammary page on www.cellntec.com to obtain further information on CELLnTEC’s mammary products.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!

Figure 23: Human primary mammary epithelium cells grown in CnT-54, after 5 population doublings (cells grown according to our recommended protocols and seeding densities)
Mammary Epithelium Cell Culture

Culture Media

By specifically targeting the progenitor cells, PCT media enable optimal isolation and colony forming efficiency, and growth, in either fully defined or low-BPE conditions, without the need for plate coating, feeder cells or other supplements. These formulations have a low calcium concentration which is necessary for cell growth without inducing differentiation.

In contrast, non-PCT media do not contain the components that specifically enhance the retention of progenitor cells. Using these media, cells can be induced to differentiate once confluent through the addition of calcium.

Isolation & Proliferation

CnT-27 - Defined, PCT medium where a completely defined environment and maximum mammary epithelial cell purity is required. The PCT formulation of CnT-27 provides excellent results through maximum retention of proliferative mammary epithelial progenitor cells.

CnT-54 - Low BPE, PCT medium recommended where the absolute best isolation efficiency, colony formation efficiency and longevity is required, and a low BPE concentration (5-8 x lower than competitors) is compatible with the experimental setup.

Differentiation

CnT-22 - Defined medium using a highly optimized basal formulation and supplements, but does not contain PCT factors. Intended for use in situations where mammary cells are induced to differentiate.

Important Note

Customized Formulations - Available on request for most media; please contact us for more details. Some standard variations are routinely available, e.g. phenol-red free, EGF free, hydrocortisone free etc.

Important Note

Protocols: Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-27</td>
<td>PCT Mammary Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CnT-54</td>
<td>PCT Mammary Epithelium Medium, Low BPE</td>
<td>Human / Mouse</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CnT-22</td>
<td>Mammary Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

Important Note

All media provided as kits containing both 500 mL basal medium and frozen supplements.

General Cell Culture Reagents: See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.

Free Samples

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSPSAMPLE</td>
<td>Mammary Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-54</td>
</tr>
</tbody>
</table>

Note: Samples include 100 mL of each medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antimycotic free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).
Melanocyte Cell Culture

Introduction

Melanocytes are neural crest-derived cells found predominantly in the basal layers of the epidermis. Melanocytes produce melanin in specialized organelles known as melanosomes. When conditions dictate, melanosomes are transferred to neighbouring keratinocytes through long protrusions known as dendrites. The melanin transferred to the keratinocytes pigments the skin, and provides protection against UV radiation.

CELLnTEC’s Progenitor Cell Targeted melanocyte medium (CnT-40) is specifically designed to enable highly efficient isolation of primary melanocyte cultures from skin tissue. It contains a variety of PCT factors that deliver both rapid proliferation and extended longevity. In addition, CnT-40 also contains a variety of specific factors that deliver high melanocyte selectivity, and retention of melanin expression during extended culture. In addition to several proprietary factors, CnT-40 contains endothelin-1, endothelin-3, alpha-MSH, and 1% FCS.

CnT-40 does not contain TPA, PMA, or cholera toxin.

Primary melanocytes growing in CnT-40 display a proliferative phenotype, in which the majority of cells exhibit 2 dendrites (see image below, left). Dendricity is significantly affected by the environment created by the culture media - for more information, please visit www.cellntec.com.

CnT-40 is also strongly selective for melanocytes, enabling highly purified cultures to be established soon after isolation (see image below, right).

For more information on primary melanocyte culture and how it can be influenced by different media components, please visit www.cellntec.com.

Proliferative primary melanocytes, isolated from adult tissue in CnT-40 medium according to the recommended protocol. Cells display a rapidly proliferating phenotype, with in general 2-3 dendrites per cell. Passage 2, day 5 post seeding.

High melanocyte selectivity. To compare the selectivity of CnT-40 (upper image) with a leading competitor DermalifeMA (lower image), an unpurified mix of melanocytes and keratinocytes isolated from adult epidermis was seeded into each medium. Six days later, the CnT-40 culture (upper) demonstrates >97% melanocyte purity, whereas the DermalifeMA culture (lower) shows significant keratinocyte contamination (cobblestone colonies).
Melanocyte Cell Culture

**Culture Media**

CELLnTEC offers a Progenitor Cell Targeted (PCT) formulation known as CnT-40 for optimal isolation and proliferation of primary melanocytes. This medium is highly selective, and will rapidly establish a pure melanocyte culture, even if some contaminating cell types are present immediately after isolation. CnT-40 does not contain TPA, PMA, or cholera toxin.

**Isolation & Proliferation**

**CnT-40** - As a result of its progenitor cell targeted (PCT) formulation and highly targeted factors, this medium offers excellent isolation and growth of primary melanocytes. It is highly selective for melanocytes. Cells growing in this medium display a proliferative phenotype, typically with 2-3 dendrites. For more information about morphology and dendrite formation, please visit www.cellntec.com.

**Important Note**

**Protocols:** Isolation, thawing, passaging, and other routine practices are significantly affected by the protocols used. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

**Special Formulations**

**Customized Formulations** - Available on request; please contact us for more details.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-40</td>
<td>PCT Melanocyte Medium</td>
<td>Human</td>
<td></td>
<td></td>
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</tbody>
</table>

**Note:** All media provided as kits containing both 500 mL basal medium and frozen supplements.

**General Cell Culture Reagents:** See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.

**Free Samples**

CELLnTEC offers the following free sample pack:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
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<tbody>
<tr>
<td>MELMSAMPLE</td>
<td>Melanocyte Media Sample Pack</td>
<td>Human</td>
<td>CnT-40</td>
</tr>
</tbody>
</table>

**Note:** Samples include 100 mL of medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antifungal free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).

**Primary Human Cells**

Primary melanocytes isolated from human skin tissue in CnT-40 (a PCT medium) demonstrate rapid growth, a proliferative morphology (mainly 2-3 dendrites per cell), and extended in vitro lifespan.

Primary cells are available from adult or juvenile donors, and are provided with a 500 mL kit of CnT-40 culture medium. The cells are also covered by our culture guarantee.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMas.05</td>
<td>Epidermal Melanocytes, Single Adult Donor (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-40</td>
</tr>
<tr>
<td>HEMns.05</td>
<td>Epidermal Melanocytes, Single Juvenile Donor (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-40</td>
</tr>
</tbody>
</table>

**Note:** All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.
Introduction

CELLnTEC’s Progenitor Cell Targeted (PCT) culture media are also available for oral epithelial cell cultivation. By specifically targeting the proliferative progenitor cells, these media offer a range of benefits including improved isolation, elevated expression of progenitor cell markers, homologous morphology and extended longevity. Publications confirm that these media are ideally suited to both human and mouse tissue.

For your convenience, primary human gingival keratinocytes (HGEP; see Figures 24 and 25) are also available. Isolated in a PCT medium, these cells provide high colony formation efficiency and excellent growth over many passages. These cells are covered by our culture guarantee, and are supplied with a 500 mL kit of PCT culture medium.

Don’t Forget!
- Please visit the oral epithelium page on www.cellntec.com to obtain independent analyses comparing our media with the competition.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!

Figure 24: Human gingival epithelium cells (HGEPp) in CnT-24, 5 days after thawing (cells grown according to our recommended protocols and seeding densities)

Figure 25: Human gingival epithelium cells (HGEPp) in CnT-24, 7 days after thawing (cells grown according to our recommended protocols and seeding densities)
Oral Epithelium Cell Culture

Culture Media

CELLnTEC offers two distinct medium formulations for oral mucosa. The Progenitor Cell Targeted (PCT) formulation known as CnT-24 offers improved isolation efficiency, marker expression, morphology and longevity. In parallel we also offer CnT-32, a non-PCT medium that is well suited for use when cells are induced to differentiate.

Isolation & Proliferation

CnT-24 - As a result of its progenitor cell targeted (PCT) formulation, this fully defined medium offers excellent isolation and growth of human oral epithelial cells. This defined medium can even equal or improve the performance of traditional non-defined media, for example those containing FBS or Bovine Pituitary Extract.

CnT-32 - Defined medium using a highly optimized basal formulation and supplements, but does not contain PCT factors. Intended for use in situations where cells are induced to differentiate. See www.cellntec.com for differentiation protocols.

Special Formulations

Customized Formulations - Available on request for most media; please contact us for more details. Some standard variations are routinely available, e.g. phenol-red free, EGF free, hydrocortisone free etc.

Important Note

Protocols: Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-24</td>
<td>PCT Oral Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CnT-32</td>
<td>Oral Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

Important Note

Protocols: Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

Free Samples

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSPSAMPLE</td>
<td>Oral Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-24</td>
</tr>
</tbody>
</table>

Free Samples

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGEPs.05</td>
<td>Gingival Epithelium Progenitors, Single Donor (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPs.15</td>
<td>Gingival Epithelium Progenitors, Single Donor (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPp.05</td>
<td>Gingival Epithelium Progenitors, Pooled (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPp.15</td>
<td>Gingival Epithelium Progenitors, Pooled (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
</tbody>
</table>

Primary Human Cells

Primary cells isolated from human gingival tissue in CnT-24 (a PCT medium) offer optimal growth, marker expression and morphology. These cells can also be induced to differentiate (see protocols for details).

Primary cells are available in two pack sizes from pooled or single donor, and are provided with a 500 mL kit of CnT-24 culture medium. The cells are also covered by our culture guarantee.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGEPs.05</td>
<td>Gingival Epithelium Progenitors, Single Donor (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPs.15</td>
<td>Gingival Epithelium Progenitors, Single Donor (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPp.05</td>
<td>Gingival Epithelium Progenitors, Pooled (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPp.15</td>
<td>Gingival Epithelium Progenitors, Pooled (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
</tbody>
</table>

Important Note

Protocols: Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

Free Samples

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSPSAMPLE</td>
<td>Oral Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-24</td>
</tr>
</tbody>
</table>

Free Samples

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGEPs.05</td>
<td>Gingival Epithelium Progenitors, Single Donor (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPs.15</td>
<td>Gingival Epithelium Progenitors, Single Donor (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPp.05</td>
<td>Gingival Epithelium Progenitors, Pooled (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
<tr>
<td>HGEPp.15</td>
<td>Gingival Epithelium Progenitors, Pooled (3 x &gt; 5 x 10⁵ viable cells)</td>
<td>Human</td>
<td>CnT-24</td>
</tr>
</tbody>
</table>

Note: All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.
**Introduction**

The prostate is comprised of epithelial glands surrounded by connective tissue. CELLnTEC’s Progenitor Cell Targeted (PCT) media create an environment that is specifically tailored to the needs of the prostate epithelium progenitor cells. In addition, our specialized fibroblast media CnT-05 (see Page 20) is also effective for the isolation and culture of the surrounding fibroblasts.

Prostate epithelium progenitors can be cultured in PCT media as adherent monolayers or as spheres. Recent publications have found that primary cells isolated in these media provide high colony forming efficiency and can proliferate over an extended period.

PCT media have also been used for the isolation and culture of tumorigenic progenitor cells from primary tumor tissue. For further details, please visit the prostate section of www.cellntec.com for further details from these publications.

CELLnTEC media can be used for the isolation of prostate epithelial progenitors from human, mouse and rat tissue. Long-term rat prostate epithelial cells are also available.

**Don’t Forget!**

- Please visit the prostate page on www.cellntec.com to obtain more information on CELLnTEC’s prostate products.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!

**Figure 26:** Human prostate epithelium cells in the defined CnT-12, after 5 population doublings (cells grown according to our recommended protocols and seeding densities)

**Figure 27:** Human prostate epithelium cells in CnT-52, low-BPE, after 5 population doublings (cells grown according to our recommended protocols and seeding densities)
**Prostate Epithelium Cell Culture**

**Culture Media**

By specifically targeting the progenitor cells, PCT media enable optimal isolation and colony forming efficiency, and growth in a fully defined environment, without the need for plate coating, feeder cells or other supplements. These formulations have a low calcium concentration that is necessary for cell growth without inducing differentiation.

In contrast, non-PCT media do not contain the components that specifically enhance the retention of progenitor cells. Using these media, cells can be induced to differentiate once confluent through the addition of calcium.

CELLnTEC provides specialized prostate epithelium media tailored to the specific needs of various species, including human, mouse, and rat.

**Isolation & Proliferation**

**CnT-12; CnT-11** - By targeting the progenitor cell population in a tissue sample, these fully defined PCT media offer significant improvements in isolation, growth and differentiation over alternative defined formulations. These defined media can even equal the performance of traditional non-defined media, for example those containing FBS.

**CnT-52** - Offers the absolute best in vitro performance by combining all the progenitor cell retention benefits of PCT, with the growth boost of BPE. The Low BPE formulation of CnT-52 contains 5 to 8 x less BPE than alternative media, thus minimising variability, and ensuring maximum accuracy and reproducibility in your experiments. When starting with small biopsies or where the absolute best isolation efficiency is required, CnT-52 is the ideal choice.

**Differentiation**

Can be done in all media when induced with i.e. calcium.

**Special Formulations**

**Customized Formulations** - Available on request for most media; please contact us for more details. Some standard variations are routinely available, e.g. phenol-red free, EGF free, hydrocortisone free etc.

**Important Note**

**Protocols:** Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>PCT</th>
<th>Defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-12</td>
<td>PCT Prostate Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CnT-52</td>
<td>PCT Prostate Epithelium Medium, Low BPE</td>
<td>Human / Mouse</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CnT-11</td>
<td>Prostate Epithelium Medium, Defined</td>
<td>Human / Mouse</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Note: All media provided as kits containing both 500 mL basal medium and frozen supplements.

**Free Samples**

CELLnTEC offers a free sample of the following medium:

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSPSAMPLE</td>
<td>Prostate Media Sample Pack</td>
<td>Human / Mouse</td>
<td>CnT-52</td>
</tr>
</tbody>
</table>

**Long-Term Animal Cells**

CELLnTEC’s long-term cell systems are your choice where the convenience of long-term growth is required. These cells have been isolated from normal tissue, and have not been actively transformed.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Species</th>
<th>Medium Included</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPROK-WIS</td>
<td>Prostate Epithelium Progenitors, Wistar (1 x &gt; 5 x 10⁵ viable cells)</td>
<td>Rat</td>
<td>CnT-11</td>
</tr>
</tbody>
</table>

**General Cell Culture Reagents:** See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.
Introduction

The vaginal mucosa features a thick, stratified, non-keratinized mucosal epithelium. Its structure is significantly affected by fluctuating levels of sex steroids, resulting in characteristic alterations in the thickness, mucification and cornification during the cycle.

Progenitor Cell Targeted (PCT) media from CELLnTEC are designed to create a precise micro-environment that actively selects and encourages growth of proliferative progenitor cells from the vaginal epithelium. As a result, these media enable efficient isolation, growth and longevity.

PCT media can be used successfully with human, mouse and rat tissue. In addition, cells isolated in a PCT medium also retain the ability to form stratified, 3D cultures (see Figure 28). For more complete information and insights from the literature, please visit www.cellntec.com.

Don’t Forget!

- Please visit the vaginal epithelium page on www.cellntec.com to obtain further information on CELLnTEC’s vaginal products.
- The extensive database on our website provides you with the option to search for methods, markers, or applications of our products from the literature.
- Publication Rewards: get a discount for every new publication you supply to us (see the publication page of our website for details).
- Newsletter: every 2 to 3 months we send out an update with all the latest developments in the literature and our product line. Stay informed, subscribe online!
Vaginal Epithelium Cell Culture

By specifically targeting the progenitor cells, PCT media enable optimal isolation, colony forming efficiency and growth in a fully defined environment, without the need for plate coating, feeder cells or other supplements. These formulations have a low calcium concentration that is necessary for cell growth without inducing differentiation.

In contrast, non-PCT media do not contain the components that specifically enhance the retention of progenitor cells. Using these media, cells can be induced to differentiate once confluent through the addition of calcium.

CELLnTEC provides specialized vaginal epithelium media tailored to the specific needs of various species, including human, mouse, and rat.

Isolation & Proliferation

**CnT-19; CnT-03** - Defined, PCT media which deliver maximum isolation efficiency and purity of human, mouse or rat vaginal epithelial cells due to the high retention of proliferative progenitor cells.

**CnT-55** - Low-BPE, PCT medium recommended where absolute best attachment, isolation and colony formation efficiency is required, the quality of the starting material is not optimal, and / or a low BPE concentration (5 to 8 x lower than competitors) is compatible with the experimental setup.

**CnT-39; CnT-33** - defined media using highly optimised basal formulations and supplements, but without PCT factors. Intended where vaginal cells are induced to differentiate.

**Special Formulations**

**Customized Formulations** - Available on request for most media; please contact us for more details. Some standard variations are routinely available, e.g. phenol-red free, EGF free, hydrocortisone free etc.

**Important Note**

**Protocols:** Isolation, thawing, passaging, differentiation and other routine practices are significantly affected by the protocols used. Serum-free media also require specific alterations to the protocols. For maximum success, please always consult and closely follow our recommended protocols found on the CELLnTEC website. For technical and scientific questions please contact scientist@cellntec.com

**Cat #** | **Description** | **Species** | **PCT** | **Defined**
---|---|---|---|---
CnT-19 | PCT Vaginal Epithelium Medium, Defined | Human / Mouse | ✓ | ✓
CnT-55 | PCT Vaginal Epithelium Medium, Low BPE | Human / Mouse | ✓ | ✓
CnT-39 | Vaginal Epithelium Medium, Defined | Human / Mouse | ✓ | ✓
CnT-03 | PCT Epidermal Keratinocyte Medium, Defined* | Rat | ✓ | ✓
CnT-33 | Epidermal Keratinocyte Medium, Defined* | Rat | ✓ | ✓

*Note: All media provided as kits containing both 500 mL basal medium and frozen supplements.

**Free Samples**

CELLnTEC offers a free sample of the following medium:

**Cat #** | **Description** | **Species** | **Medium Included**
---|---|---|---
VMSPSAMPLE | Vaginal Media Sample Pack | Human / Mouse | CnT-55

*Note: Samples include 100 mL of each medium and are completely supplemented. They are ready to use – no need for additional components. They are antibiotic / antifungal free and have a shelf life of 8 weeks after production (stored at 4°C in the dark).

**Long-Term Animal Cells**

CELLnTEC’s long-term cell systems are your choice where the convenience of long-term growth is required. These cells have been isolated from normal tissue, and have not been actively transformed.

**Cat #** | **Description** | **Species** | **Medium Included**
---|---|---|---
RPVAK-WIS | Vaginal Epithelium Progenitors, Wistar (1 x > 5 x 10⁶ viable cells) | Rat | CnT-03

*Note: All cells are provided as a kit with 500 mL basal medium and frozen supplements, plus guaranteed longevity.

**General Cell Culture Reagents:** See Page 32 for additional products, including enzymes, freezing media, antibiotics, BPE, and stains.
## Antibiotics / Antimycotics

CELLnTEC provides two antibiotic alternatives, namely traditional penicillin / streptomycin, or gentamycin.

Gentamycin is an increasingly favored antibiotic, due to its better thermo stability, acidic resistance, broader effective spectrum, and longer shelf life at 4°C in the dark. For a more detailed comparison of these products, please visit www.cellntec.com.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Pack Size</th>
<th>Sufficient for</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-GAB10</td>
<td>Gentamycin / Amphotericin B Solution, 500 x, Ready-to-Use Single Aliquots</td>
<td>10 x 1 mL</td>
<td>10 x 500 mL bottles</td>
</tr>
<tr>
<td>CnT-ABM10</td>
<td>Pen / Strep / Amphotericin B Solution, 200 x, Ready-to-Use Single Aliquots</td>
<td>10 x 2.5 mL</td>
<td>10 x 500 mL bottles</td>
</tr>
</tbody>
</table>

## Freezing Medium

Formulated using only synthetic compounds, the xeno-free CRYO-Defined is an advanced new freezing medium that is fully defined, and thus no longer depends on the use of variable additives such as serum.

In testing this medium has been found to deliver equal or better cell recovery after thawing than traditional high-serum freezing media (see Figure 29 for a comparison).

![Serum-Based Freezing Medium](image1)

![Cryo-Defined Freezing Medium](image2)

**Figure 29:** Growth test after thawing, comparing serum-based medium (Left) with CRYO-Defined (Right). Human epidermal keratinocytes, day 8 after thawing.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Pack Size</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-CRYO-50</td>
<td>CRYO-Defined, Animal Component Free Freezing Medium</td>
<td>50 mL</td>
<td>2 x</td>
</tr>
</tbody>
</table>

## Bovine Pituitary Extract (BPE)

BPE is a well known non-defined cell culture supplement, which contains growth factors and has a beneficial effect on attachment and growth of primary cells and cell lines. CELLnTEC’s media do not require any additional supplements. However, in other situations where an optimized medium is not available, the cells of a specific species are difficult to culture or the quality of the starting tissue sample for isolation is not optimal, you may wish to evaluate an additional BPE supplementation.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Pack Size</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-BPE-5</td>
<td>Bovine Pituitary Extract (BPE)</td>
<td>5 x 1 mL</td>
<td>New Zealand</td>
</tr>
</tbody>
</table>
General Cell Culture Reagents

Accutase Detachment Solution

Accutase® has been developed to meet the most demanding needs for gentle and effective detachment of adherent cells and can be used as a direct replacement for trypsin solution. It is useful for the routine detachment of cells from standard tissue culture plastic ware and adhesion coated plastic ware, and has been proven effective in detaching fibroblasts, epithelial cells and a whole range of other cell types. Unlike trypsin, it is gentle, and is stopped simply by dilution (no separate reagent required to stop the reaction).

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-Accutase-100</td>
<td>Accutase Cell Detachment Solution</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

Dispase (Neutral Protease)

Dispase® is a neutral protease which cleaves fibronectin, collagen IV, and to a lesser extent collagen I. It is widely used in epithelial cell culture to separate epithelial tissues from the underlying mesenchyma, thereby avoiding the need to seed mixed epithelial and mesenchymal cell populations in primary culture.

Dispase is a gentle enzyme, that can be used either at 4°C overnight, of alternatively at room temperature for several hours. It is most commonly used during isolation, but can also be used to passage sensitive cell-types. For more detailed protocol info, please visit www.cellntec.com.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-DNP-10</td>
<td>Dispase II, Neutral Protease</td>
<td>10 mL, 20x solution</td>
</tr>
</tbody>
</table>

Stain Solution Kit

The stain kit can be used to stain fixed cells. It consists of two different ready-to-use components which can be re-used up to five times. The stain kit can be used for cells growing on different surfaces:

- Cell culture inserts. The stain solutions can be used on polycarbonate and PET membranes. The cells can be stained on mixed cellulose ester membranes, only when ethanol is utilizes as a fixing agent.
- Cells growing on cell culture plastic surface can be stained to monitor for colony forming efficiency, growth pattern etc.
- Cells growing on chamber slides.

For staining procedures, please see the general staining protocol in the resources section of www.cellntec.com.

<table>
<thead>
<tr>
<th>Cat #</th>
<th>Description</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>CnT-ST-100</td>
<td>Stain Kit, including Solution I and Solution II</td>
<td>2 x 50 mL</td>
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</table>
Precise protocols are an integral part of successful cell culture. They result from significant research and development, and must consider both the biological needs of the cells and the physical properties of the various reagents.

CELLnTEC provides an extensive array of standard and specialty protocols on www.cellntec.com. A summary of the protocols available can be found below.

### Protocols

CELLnTEC’s protocols cover a broad range of techniques, including isolation, passaging / freezing, transfection and differentiation. The table below gives a short overview of CELLnTEC’s existing recommended protocols per tissue type:

#### General Cell Culture Protocols

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Species</th>
<th>Isolation</th>
<th>2D Cultivation</th>
<th>2D Differentiation</th>
<th>3D Protocols</th>
<th>Seeding Densities</th>
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<td>✓</td>
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</tbody>
</table>

**Note:** * see also our instructional 3D Epidermal Cell Culture Video

#### Specialty Protocols

Other useful protocols that are also available include:
- Routine Histology and Staining of 3D Cell Cultures
- OCT Embedding of 3D Cultures for Cryo-Sectioning
- Keratinocyte Transfection Using PEI
- Staining of Cells Grown in 2D
- Aging of Keratinocytes using CnT-AG2
- Establishment of hanging-drop spheroid cultures
- Lipid Extraction

### FAQ’s / Glossary

For your convenience the resources section on the CELLnTEC website provides answers to a range of frequently asked questions (FAQs) as well as a comprehensive glossary, explaining some of the most important terms used within CELLnTEC and in general epithelial cell culture.

### Publications / References

A searchable data base with scientific publications describing the use of CELLnTEC’s products can also be found online.

www.cellntec.com
Contract Research and Testing

CELLnTEC is a research-based company that uses detailed biological insights to design novel 2D and 3D in vitro models and testing methods. Our extensive experience in the design and completion of challenging in vitro experiments is now routinely used by the biotech, pharma and cosmeceutical industries.

Experience has shown that powerful new insights can regularly be obtained using models and methods that are specifically tailored to the requirements of a particular investigation. Customized approaches to challenging questions are our specialty, for more information about our contract research services, please contact services@cellntec.com

Cosmeceutical

The cosmetic industry is driven by the need for novel claims that enable cutting edge products to stand out from the crowd. In addition to a range of standard testing methods, we specialize in the development of novel approaches that deliver unique claims to differentiate your product in the market.

Our focus on new approaches has lead us to create exciting new progenitor and stem cell-based methods, as well as anti-aging models in 2D and 3D, plus a host of other protocols to quantify novel mechanisms and end-points. We work hard to be the best scientific partner for your in vitro testing needs - don’t hesitate to contact us and ask what methods would be best suited to your next project.

Biotech and Pharma

CELLnTEC’s precision 2D and 3D models are ideally suited for investigations in the fields of toxicology, drug testing and cancer stem cells. We have extensive experience with human and rodent tissues, and are able to provide a range of testing panels featuring several tissues and species.

With a focus on highly-accurate modeling, our contract research services are specifically designed to deliver more predictive and deeper insights into biological processes. With tailored models covering the full spectrum from undifferentiated progenitors through to fully differentiated 3D models, we are able to deliver new possibilities in the fields of predictive and mechanistic toxicology, screening of new leads in accurate untransformed models, as well as detailed investigation of progenitor and stem cells isolated from normal and tumor tissue.

Figure 30: Above: Formation of an intact 3D structure by epidermal progenitor cells protected from aging by a cosmetic active
Below: Aged epidermal progenitor cells lose the capacity to form 3D normal structure

Figure 31: Top - Primary rat bladder epithelium, isolated in CnT-58, differentiated in 3D for 18 days in CnT-21 medium and calcium. Bottom - same model stained for uroplakin
**How To Order**

CELLnTEC provides its products directly to a range of countries in Europe, including France, Germany, Italy, Switzerland and Scandinavia. For distributors in other countries worldwide, please see the following pages, and www.cellntec.com for the latest info.

If you would like to order direct from CELLnTEC, please see the info below. For other countries, the distributor for your region will be pleased to provide you with detailed ordering and pricing info.

**Orders Direct to CELLnTEC**

**General Information**

CELLnTEC has established a fast and reliable shipping system which enables us to deliver our products to all major cities in all EU member state countries as well as Switzerland within 24 hours. Orders are shipped Tuesdays and Wednesdays and, when placed by 8.30 am Tuesday and Wednesday, will ship the same day (subject to availability).

For most rapid processing, please submit orders by email to orders@cellntec.com or fax (+41 31 331 9583).

Our price lists are available online on our website in the ordering section (www.cellntec.com/orders). If you are a regular customer or are interested in ordering larger volumes, please contact us for a quote.

If you need any further assistance, feel free to contact us either directly by phone or by sending an email to orders@cellntec.com

**Terms and Conditions**

Selected elements of CELLnTEC’s terms and conditions of sale are described below. All transactions are subject to the complete list of “General Terms and Conditions” published on www.cellntec.com.

Conditions of purchase provided by the customer shall have no validity, even when not expressly described or contradicted.

By submitting their order and accepting delivery of the goods, the customer is deemed to have agreed to these conditions of sale and delivery. Any additional conditions or terms of sale proposed by the customer shall only become valid following our express written confirmation.

Prices found in the official Price List are net prices, excluding any applicable taxes, duties and shipping costs. Payment Terms are net 30 days.

All risk for loss or damage passes to the customer upon collection of the goods by the carrier at our facility, shipping terms are FCA Origin. Complaints must be made immediately in writing. In case of faulty products, goods will be replaced at our cost. Claims for compensation are excluded.

Goods are to be used only in the ways specifically stated in the corresponding product literature. Liability for improper product use is expressly declined.

For complete General Terms and Conditions, please visit www.cellntec.com

**Distributors**

For the latest list of distributors in the Americas, Europe, and Asia, please visit www.cellntec.com.

A summary of key distribution partners can also be found on pages 37 - 39 of this catalog.
Ordering North America

CELLnTEC products are available throughout North America from one of our distributors. To find your local vendor, please see below. For other countries, please see the following pages or www.cellntec.com.

United States of America

In the USA, CELLnTEC products are available from our partners at Zen-Bio:

Zen-Bio Inc.
Phone: 1-866-234-7673
Fax: 1-919-547-0693
information@zen-bio.com
www.zen-bio.com

Canada

In Canada, products are available from CEDARLANE:

Cedarlane Laboratories Ltd.
Phone: 1-800-268-5058
Fax: 1-289-288-002
custserv@cedarlanelabs.com
www.cedarlanelabs.com
Ordering Europe

In Europe, CELLnTEC products may be ordered either direct from CELLnTEC, or from one of our distributors.

For the latest info on ordering, pricing, and new distributors, please visit www.cellntec.com.

Orders Direct to CELLnTEC

Customers in the following countries can order direct from CELLnTEC (see page 36):

- France
- Germany
- Italy
- Scandinavia
- Switzerland
- Austria
- Eastern Europe

CELLnTEC Advanced Cell Systems AG
Phone: +41 (0)31 331 9582
Fax: +41 (0)31 331 9583
Email: orders@cellntec.com
Web: www.cellntec.com

Distributors Europe

In the following countries, products can also be ordered from one of our distribution partners:

Benelux
Bio-Connect B.V.
Phone: +31 (0)26 326 4450
Fax: +31 (0)26 326 4451
Email: orders@bio-connect.nl
Web: www.bio-connect.nl

Spain and Portugal
Genycell Biotech España S.L.
Phone: +34 902 194 353
Fax: +34 958 513 149
Email: sales@genycell.com
Web: www.genycell.com

Italy
SIAL Group
00167 Roma
Phone: +39-06-6629818
Web: www.sialgroup.com

Scandinavia
AH diagnostics
Web: www.ahdiagnostics.dk / fi / se / no
Please visit their website for your country’s order details.
Further information can be obtained on www.cellntec.com

UK and Ireland
Caltag Medsystems Ltd
Phone: 01280 827460
Fax: 01280 827466
Email: office@caltagmedsystems.co.uk
Web: www.caltagmedsystems.co.uk
## Distributors Worldwide

### Malaysia
Team Medical & Scientific  
Phone: +603-5122 5108  
Fax: +603-5122 1608  
Email: info@tms-lab.com  
Web: www.tms-lab.com

### Singapore
Bio-Rev Pte Ltd  
Phone: (65) 6273 3022  
Fax: (65) 6273 3020  
Email: sales@bio-rev.com  
Web: www.bio-rev.com

### South Korea
Koma Biotech  
Phone: 82-2-579-8787  
Fax: 82-2-578-7042  
Email: koma@komabiotech.co.kr  
Web: www.komabiotech.co.kr

### Dubai / Middle East
Ameg Tech FZC  
Dubai UAE  
Email: info@ameg.ae  
Web: www.ameg.ae

### Japan
Funakoshi Co Ltd  
Phone: +81-3-5684-1620  
Email: reagent@funakoshi.co.jp  
Web: www.funakoshi.co.jp

### China
Premedical Laboratories Inc  
Beijing  
Phone: 010-51666388-8003  
Email: premedlab@gmail.com  
Web: www.premedlab.com

### India
Mount Biosciences  
Hyderabad  
Web: www.mountbio.com

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Email: orders@cellntec.com  
Web: www.cellntec.com

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Protocols See the resources page on the website
Website www.cellntec.com