

# Mammary Epithelium Isolation

This document describes the recommended protocol for isolation of primary human mammary epithelial cells using CnT-Prime (CnT-PR) medium.

This protocol suggests the seeding of small cell clumps/organoids directly after dispase/collagenase separation, thereby avoiding the additional digestion step to produce single cells.

For the recommended thawing, passaging, and freezing protocols, please see the General Cultivation Protocol in the resources section of [www.cellntec.com](http://www.cellntec.com).

## Preparation

Before starting to work with the cells, ensure that any medium, buffers or enzymes required have been prepared at the recommended concentrations.

Always use sterile instruments, aseptic technique, and work in a laminar flow to maintain sterility.

## Isolation Protocol

1. Cut mammary tissue sample into small pieces, remove excess fat
2. Incubate overnight at 37°C in CnT-PR with 1x dispase (CnT-DNP-10) + 500 µg / mL collagenase 2 + 2 x antibiotic / antimycotic (CnT-GAB-10)
3. Vigorously pipette or vortex to break up clumps
4. Centrifuge 5 min 1000 rpm, wash until all the fat is removed
5. Pass through 40micron mesh filter (cell strainer), wash filter with 10 mL medium. Reverse wash organoids off filter into new tube
6. Wash epithelial organoids once or twice with medium
7. Seed organoids directly into plate (recommended), or if single cells are required, digest organoids with Accutase\* before seeding cells at  $2 \times 10^4$  per  $\text{cm}^2$ .
8. Culture cells in CnT-PR supplemented with IsoBoost (CnT-ISO-50) for at least the first 3 days post seeding, then switch to standard CnT-PR medium. Change medium every 2-3 days after cells have attached. Passage cells before they reach confluency, using Accutase (CnT-Acctuase-100) for 10-15 min at 37°C. Recommended seeding density after passaging is 4'000 cells per  $\text{cm}^2$

For additional culture protocols (passaging, freezing, thawing) please visit the resources section of [www.cellntec.com](http://www.cellntec.com).

In case of questions, please email out scientists directly: [scientist@cellntec.com](mailto:scientist@cellntec.com)