ROCKLAND™
antibodies & assays

Cellaria

Using WIT Culture Media for BPEC Culture
Protocol

I. Materials

Product
WIT-P Culture Medium (CM-0101) or WIT-P-NC Culture Medium (CM-0104)
WIT-T Culture Medium (CM-0103)
Cholera Toxin (EMD Millipore 227036 recommended)
Collagenase A (Roche 11088885103 recommended)
Primaria™ Cell Culture Flask 25 cm
Hank's Buffered Salt Solution (HBSS)
Trypsin

II. Preparation of Complete WIT Medium

1. Thaw the vial of WIT supplement in a 37°C water bath.
2. In a sterile environment, add the entire contents of the WIT supplement vial to the bottle of WIT Basal Medium.
3. If using WIT-P-NC, add Cholera Toxin to a final concentration of 100 ng/mL
   Note: Protection should be worn when handling Cholera Toxin or supplement vials for WIT-P media. Any media spills should be cleaned immediately and environments should be wiped down (70% ethanol and bleach) after working with either media.

III. Preparation of Breast Primary Epithelial Cells (BPECs)

1. Using a sterile scalpel in a sterile environment, mince human mammary tissue on ice to 1mm^3 pieces.
2. Transfer minced tissue to a 15 mL conical tube and add 1 mg/mL Collagenase A in Hank's Buffered Salt Solution (HBSS). With a sterile pipette, mix the solution until the tissue is well suspended. Allow the tissue to dissociate in this solution at 37°C for 6–8 hours with constant slow rotation.
3. After dissociation, transfer the mixture to polypropylene conical tubes and centrifuge at 10 x g for 5 minutes.
4. Remove and discard the fat layer above the pellet. Resuspend the pellet in an appropriate amount of WIT-P medium. Plate at approximately 10–20 organoids per cm^2 in Primaria™ Cultureware. Each culture dish should contain 10–20 mL of medium per 25 cm^2 of plate surface area.
   Note: Do not dissociate the organoids to single cells using trypsin digestion or other mechanical or enzymatic methods. Such treatment will severely reduce the number of isolated BPEC colonies. Also, the use of Primaria™ Cultureware is critical for the culture of all types of breast epithelial cells.
5. Culture the sample for an additional 10–15 days at 37°C and 5% CO_2 while changing the WIT-P medium every 2 days. Use 10–20 mL of medium per 25 cm^2 of plate surface area.

IV. Culture and Subculture of Cells in WIT (WIT-P or WIT-T) Media

1. Maintain cultures on Primaria™ Cultureware plates at 37°C and 5% CO_2.
2. The WIT-P culture media should be changed for BPECs every 2 days with WIT-P medium or transformed derivatives every 3 days with WIT-T medium.
3. When the culture is 75–95% confluent, it should be passaged.
4. Dissociate cells by treatment with 0.15% trypsin at 37°C for no more than 2–3 minutes. Dislodge cells from the plate surface by shaking or gently knocking the plate against your hand.
   Note: Do not use cell scrapers to remove cells from the plate.
5. Inactivate the trypsin by adding 10 mL of the appropriate WIT medium supplemented with 20% fetal bovine serum for every 1 mL of 0.15% trypsin used.
6. Using polypropylene centrifuge tubes centrifuge the cells at 500 x g for 5 minutes and remove the supernatant. Add a sufficient amount of WIT medium to resuspend the cells for counting.
   Note: Use of polypropylene tubes for pelleting BPECs (and their derivatives) is critical because these cells will not form a pellet when they are centrifuged in other types of plastic tubes such as polystyrene.
7. Re-plate cells in the appropriate WIT medium at a density of 1 x 10^4 cells per cm^2. Continue to culture at 37°C and 5% CO_2.
   Note: Do not re-plate cells at densities less than 7.5 x 10^3 cells per cm^2 or more than 1.5 x 10^4 cells per cm^2.
8. Change the medium 24 hours after plating and every 2 days thereafter.
   Note: It is common to observe some dead cells in the first 24–48 hours after subculturing.