



PROTOCOLS Using Cellaria WIT (WIT-P, WIT-P-NC, and WIT-T) Culture Media for BPEC Culture

Materials

- Cellaria WIT-P Culture Medium (Cellaria Cat. No. CM-0101) or WIT-P-NC Culture Medium (Cellaria Cat. No. CM-0104)
- Cellaria WIT-T Culture Medium (Cellaria Cat. No. CM-0103)
- Cholera Toxin, *Vibrio cholerae*, Type Inaba 569B (EMD Cat. No. 227036)
- Collagenase A (Roche Cat. No. 11088785103)
- Primaria™ Cell Culture Flask 25 cm (Corning Cat. No. 353808)

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 (Cellaria Cat. No. CM-0104) add cholera toxin at the following concentrations:
WIT-P-NC: Add cholera toxin to a final concentration of 100 ng/ml
Note: When handling cholera toxin, the supplement vials For WIT-P media, or when performing cell culture using medium with cholera toxin, gloves, lab coat and eye protection should be worn. Any media spills should be cleaned immediately. Cell culture environments should be wiped down (70% ethanol and bleach) after completing work with either of these media.

Preparation of Breast Primary Epithelial Cells (BPECs)

1. Using a sterile scalpel in a sterile environment, on ice, mince human mammary tissue to 1mm³ 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 to 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 to 20 organoids per cm² in Primaria™ Cultureware. Each culture dish should contain between 10 ml and 20 ml of medium per 25 cm² 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.



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5. Culture the sample for an additional 10 to 15 days at 37°C and 5% CO², changing the WIT-P medium every 2 days. Use between 10 ml and 20 ml of medium per 25 cm² of plate surface area.

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% CO².
2. The WIT-P culture media should be changed:
 - A. BPECs: every 2 days with WIT-P medium.
 - B. Transformed derivatives: every 3 days with WIT-T medium.
3. When the culture is 75 to 95% confluent, it should be passaged.
4. Dissociate cells by treatment with 0.15% trypsin at 37°C for no more than 2 to 3 minutes. Dislodge cells from the plate surface by shaking or gently knocking the plate against your hand. 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 the cells in the appropriate WIT medium at a density of 1 x 10⁴ cells per cm², and continue to culture at 37°C and 5% CO².

Note: Do not re-plate cells, particularly BPECs, at densities less than 7.5 x 10³ cells per cm², or more than 1.5 x 10⁴ cells per cm².
8. Change the medium 24 hours after plating, and thereafter every 2 days.

Note: It is common to observe some dead cells in the first 24-48 hours after subculturing.

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For Technical Support, call 1-617-981-4208.

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