Tissue Dissociation Flask

Borosilicate glass flat-bottom flask for enzymatic digestion of tissue

Catalog # 27300 1 Flask

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Product Description

Borosilicate glass flat-bottom 250 mL flask with break-resistant lip. Designed for the enzymatic digestion of tissue fragments and homogenates into cell suspensions.

Properties

Storage: Store at 15 - 25°C.
Shelf Life: Not applicable

Handling / Directions For Use

EXAMPLE: Dissociation of human mammary tissue

For more information on the dissociation of human mammary tissue, refer to the Product Information Sheet for EpiCult™-C Human Medium Kit (Document #29967) available at www.stemcell.com or contact us to request a copy.

NOTE: Avoid the use of glass pipettes and tubes when handling mammary epithelial cells, as these cells will stick to glass.

1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in DMEM/F-12 with 15 mM HEPES (Catalog #36254) supplemented with 5% fetal bovine serum (FBS).
2. Transfer tissue to sterile glass Petri dishes, mince with scalpels and then transfer to tissue dissociation flasks.
   NOTE: Glass Petri dishes can be used for this initial dissociation, as the concentration of epithelial cells is very low.
3. Dilute 1 part Collagenase/Hyaluronidase (Catalog #07912) with 9 parts complete EpiCult™-C Medium (Human; Catalog #05630) and add to the minced tissue in the dissociation flasks.
   NOTE: Alternatively, mammary tissue can be dissociated in DMEM/F-12 with 15 mM HEPES supplemented with 2% w/v Fraction V BSA, to avoid influences of exogenous growth factors and FBS; however, this may result in lower total viable cell yields.
4. Ensure that the tissue is well-suspended in the enzyme mixture and the final volume is level with the widest portion of the flask. Cover the opening of the flask with sterile aluminum foil.
5. Gently dissociate the minced tissue on a rotary shaker at 37°C until all large tissue fragments are digested. Typical digestion time is 16 hours (overnight) for normal human mammary tissue. Longer digestion times may be required for tough fibrous tissue, shorter digestion times for softer tissue.
   NOTE: The flasks should be sealed with Parafilm® if the rotary shaker is not in a 5% CO_2 incubator.
6. Transfer the dissociated tissue to 50 mL conical tubes (e.g. Catalog #38010), and centrifuge at 80 x g for 30 seconds.
7. Discard the overlying liquefied fat layer. The pellet (“A” pellet) is highly enriched for terminal ductal lobular unit (TDLU) epithelial fragments.
8. Transfer the supernatant to a new 50 mL conical tube and centrifuge at 200 x g for 3 minutes. The pellet (“B” pellet) from this second centrifugation contains variable numbers of epithelial cells, stromal cells, and red blood cells.
9. The supernatant from the second centrifugation is enriched for human mammary fibroblasts. To collect, transfer the supernatant to a new 50 mL conical tube and centrifuge at 350 x g for 5 minutes.
10. The different cell fractions can now be cryopreserved. It is recommended that cells are cryopreserved in complete EpiCult™-C Medium (Human) supplemented with 50% FBS and 6% dimethyl sulfoxide (DMSO).