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™-B Medium (Human; Document #29567) available on our website at www.stemcell.com or contact us to request a copy.

1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in complete Epicult™-B Medium (Human; Catalog #05601) supplemented with 5% fetal bovine serum (FBS).
2. Transfer the 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™-B Medium and add to the minced tissue in the dissociation flasks. 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.
4. Dissociate minced tissue for approximately 16 hours (overnight) on a rotary shaker at 37°C. Longer dissociation times may be required for tough, fibrous tissue, shorter digestion times for softer tissue.
   NOTE: The flasks should be sealed if the rotary shaker is not in a 5% CO_2 incubator.
5. After dissociation, transfer the tissue to 50 mL centrifuge tubes, and centrifuge at 80 x g for 30 seconds.
6. Discard the overlying liquefied fat layer. The pellet (“A” pellet) is highly enriched for epithelial organoids.
7. Transfer the supernatant to a new 50 mL centrifuge 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.
8. The supernatant from the second centrifugation is enriched for human mammary fibroblasts. To collect, transfer the supernatant to a new 50 mL centrifuge tube and centrifuge at 350 x g for 5 minutes.
9. The different cell fractions can now be cryopreserved, or a single-cell suspension can be generated with additional processing.