Collagenase/Hyaluronidase

10X Collagenase/hyaluronidase in DMEM

Catalog # 07912 10 mL

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Product Description

10X Collagenase/Hyaluronidase in Dulbecco's Modified Eagle's Medium (DMEM) for the enzymatic dissociation of human mammary tissue.

Properties

Storage: Store at -20°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:
- 3000 U/mL Collagenase
- 1000 U/mL Hyaluronidase
- DMEM (1000 mg D-glucose/L)

Materials Required But Not Included

- Ammonium Chloride Solution (Catalog #07800)
- EpiCult™-C Human Medium Kit (Catalog #05630)
- HBSS with 10 mM HEPES, Without Phenol Red (Catalog #37150)
- Tissue Dissociation Flask (Catalog #27300)
- DMEM/F-12 with 15 mM HEPES (Catalog #36254)

Handling / Directions For Use

Thaw Collagenase/Hyaluronidase at room temperature (15 - 25°C) or overnight at 2 - 8°C.
NOTE: Collagenase/Hyaluronidase is provided as a 10X stock solution, and should be diluted 1 in 10 as outlined in the protocol below. Once thawed, use immediately or aliquot and store at -20°C until the expiry date as indicated on the label. After thawing aliquots, use immediately. Do not re-freeze.

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

DISSOCIATION OF HUMAN MAMMARY TISSUE

1. Transport human mammary tissue from the operating room on ice in sterile specimen cups in DMEM/F-12 with 15 mM HEPES 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 (10X stock) with 9 parts complete EpiCult™-C Medium (Human) 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).

To generate a single-cell suspension from the “A” or “B” pellet, 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.