Gentle Collagenase/Hyaluronidase

10X Gentle collagenase/hyaluronidase in DMEM

Catalog # 07919 10 mL

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Product Description

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

Properties

Storage: Store at -20°C.

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

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

Materials Required But Not Included

- Ammonium Chloride Solution (Catalog #07800)
- EpiCult™-B Mouse Medium Kit (Catalog #05610)
- Fetal bovine serum (FBS)
- HBSS with 10 mM HEPES, Without Phenol Red (Catalog #37150)
- Gentamicin

Handling / Directions For Use

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

DISSOCIATION OF MOUSE MAMMARY TISSUE

1. Thaw Gentle Collagenase/Hyaluronidase at room temperature (15 - 25°C) or overnight at 2 - 8°C.
   NOTE: Once thawed, use immediately or aliquot and store at -20°C until the expiry date as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.
2. Dilute Gentle Collagenase/Hyaluronidase as outlined below for either 2-hour or overnight dissociation.
   NOTE: Approximately 2 - 5 mL of the EpiCult™-B Medium/Collagenase/Hyaluronidase/FBS/gentamicin solution will be required for every 2 mammary glands to be dissociated. Alternatively, mammary glands can be dissociated in DMEM/F-12 with 15 mM HEPES (Catalog #36254) supplemented with 50 µg/mL gentamicin to avoid influences of exogenous growth factors and FBS; however, this may result in lower total viable cell yields.

   2-HOUR DISSOCIATION
   Dilute 1 part Gentle Collagenase/Hyaluronidase with 4 parts complete EpiCult™-B Medium (Mouse) supplemented with 5% FBS and 50 µg/mL gentamicin in a 15 mL or 50 mL tube (e.g. Catalog #38009 or 38010). Continue to step 3.

   OVERNIGHT DISSOCIATION
   Dilute 1 part Gentle Collagenase/Hyaluronidase with 9 parts complete EpiCult™-B Medium (Mouse) supplemented with 5% FBS and 50 µg/mL gentamicin in a 15 mL or 50 mL tube (e.g. Catalog #38009 or 38010). Continue to step 4.

3. For 2-hour dissociation, resect mammary glands and transfer to a sterile glass Petri dish. Mince with scalpels in a crosswise pattern until glands are rendered to a paste. This step is not necessary for overnight dissociation.
   NOTE: It is essential that the glands are well minced, or total viable epithelial cell yield will be low.

4. Transfer the mammary tissue to the tube containing EpiCult™-B Medium /Collagenase/Hyaluronidase/FBS/gentamicin.

   2-HOUR DISSOCIATION: Incubate at 37°C on a rotary/rocking shaker set at 90 rpm for 2 hours. After 1 hour and at the end of incubation, pipette the suspension up and down 20 times using a 1 mL pipettor.

   OVERNIGHT DISSOCIATION: Incubate at 37°C for 15 hours (overnight). It is not necessary to use a shaker.

5. Centrifuge the cells at 350 x g for 5 minutes and discard the supernatant.
6. Resuspend the cell pellet in a mixture of 1 part cold HBSS with 10 mM HEPES, Without Phenol Red supplemented with 2% FBS and 4 parts Ammonium Chloride Solution and centrifuge at 350 x g for 5 minutes. The resultant pellet contains epithelial cell organoids as well as stromal cells and lymphocytes.

To generate a single-cell suspension of mammary epithelial cells, refer to the Technical Bulletin: Monolayer Culture of Mouse Mammary Epithelial Cells (Document #29179), available at www.stemcell.com or contact us to request a copy.

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