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Gentle Collagenase/Hyaluronidase

10X Gentle collagenase/hyaluronidase in DMEM

Catalog #07919 10 mL

Product Description

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

Properties

Stability and Storage: Store at -20°C. Stable until expiry date (EXP) on label.

Contains:

- 10 mg/mL Collagenase (> 1.5 U/mL)
- 1000 U/mL Hyaluronidase
- DMEM (1000 mg D-glucose/L)

Directions for Use

The following materials are 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

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. 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.

Warning

This product is hazardous. Please refer to the Safety Data Sheet (SDS).

Notes and Tips

To generate a single-cell suspension of mammary epithelial cells, refer to the Product Information Sheet for MammoCult™ Organoid Kit (Mouse; Document #10000036552), available at www.stemcell.com, or contact us to request a copy.

Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www. stemcell.com/hPSCworkflow, or contact us at techsupport@stemcell.com.

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