Gentle Collagenase/Hyaluronidase



Catalog # 07919 10 mL



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FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES

Product Description

10X Collagenase/Hyaluronidase in Dulbecco's Modified Eagle's Medium (DMEM). Recommended for the overnight 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 HyaluronidaseDMEM (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 for Human Myeloid Colony-Forming Cells (or equivalent) (Catalog #06100)
- HBSS with 10 mM HEPES, Without Phenol Red (Catalog #37150)

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: Gentle 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 the aliquots, use immediately. Do not re-freeze.
- 2. Dilute 1 part Gentle Collagenase/Hyaluronidase (10X stock) with 9 parts complete EpiCult™-B Medium (Mouse) supplemented with 5% FBS in a 14 mL or 50 mL tube.
 - NOTE: Approximately 2 5 mL of the EpiCultTM-B Medium/Collagenase/Hyaluronidase/FBS solution will be required for every 2 mammary glands to be dissociated.
- 3. Resect mammary glands and transfer to a sterile glass petri dish. Mince with scalpels in a cross-wise pattern until glands are rendered to a paste. Transfer the mammary tissue to the tube containing EpiCult™-B Medium/ Collagenase/Hyaluronidase/FBS and incubate at 37°C for 15 hours (overnight).
- 4. Centrifuge the cells at 350 x g for 5 minutes and discard the supernatant.
- 5. Resuspend the cell pellet in a mixture of 1 part cold Hanks' Balanced Salt Solution (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 on our website at www.stemcell.com, or contact us to request a copy.

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